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(57) Abstract

This invention provides an isolated nucleic acid molecule encoding a SEL-12. This invention further provides an isolated nucleic acid molecule which encodes a mutated SEL-12. This invention also provides an isolated nucleic acid molecule which encodes a mutated SEL-12, wherein the mutated SEL-12 contains at least one of the following: position 115 is a leucine, position 132 is an arginine, position 215 is a glutamic acid, position 229 is a valine, position 254 is a valine, position 255 is a valine, position 371 is a valine, position 387 is a tyrosine, position 104 is an isoleucine or position 204 is a valine. This invention further provides different uses of these nucleic acid molecules. This invention also provides different *sel*-12 mutants and transgenic animals which carry wild-type or mutated *sel*-12.

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reducing or eliminating *sel-12* activity causes and egg-laying defective (Egl) phenotype. Applicants do not know if the Egl phenotype is a direct consequence of reducing *lin-12* activity or an independent effect of reducing *sel-12* activity. (2) *sel-12* and *lin-12* can functionally interact within the same cell. (3) *sel-12* is predicted to encode a protein with multiple transmembrane domains that is highly similar to S182, which has been implicated in early-onset familial Alzheimer's disease (Sherrington et al., 1995). These findings have been described in a paper that has been accepted by *Nature* (Levitan and Greenwald, 1995). In addition, applicants have data indicating that *sel-12* is more broadly expressed than *lin-12*, including a lot of expression in neurons.

The remarkable conservation of the SEL-12 and S182 predicted protein structure suggests that their functions are likely to be conserved as well. Recently, a second gene known as E5-1 or STM2 has been implicated in early-onset familial Alzheimer's disease (Levy-Lahad et al, 1995; Rogaev et al, 1995) E5-1/STM2 encodes a protein that is highly similar to S182 (Levy-Lahad et al, 1995b; Rogaev et al, 1995) and SEL-12. Furthermore, it is striking that four of the five changes in S182 or E5-1/STM2 associated with early-onset familial Alzheimer's disease alter amino acids that are absolutely conserved in the worm and the human proteins, and that the tenth alters an amino acid that has been changed very conservatively during evolution. Applicants hope to bring the powerful tools of classical and molecular genetic studies in *C. elegans* to bear on fundamental issues of SEL-12/S182/E5-1 structure and function. Thus, far, proteins similar to LIN-12 and SEL-12 have not been described in single-celled organisms, so *C. elegans* may be the simplest practical system for studying these issues in vivo.

IDENTIFICATION OF *sel-12* AND USES THEREOF

This application claims benefit of U.S. Provisional Application No. 60/004,387, filed September 27, 1995, the content of which is incorporated into this application by reference.

Within this application, publications are referenced within parentheses. Full citations for these references may be found at the end of each series of experiments. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

Background of the Invention

The *lin-12* gene of *C. elegans* is the archetype of the "*lin-12/Notch*" gene family found throughout the animal kingdom (reviewed in Greenwald and Rubin, 1992). Members of this family appear to function as receptors for intercellular signals that specify cell fates during development. Essentially, *lin-12* activity controls binary decisions: if a cell has a choice between two fates, A and B, activation of *lin-12* above a threshold value causes the cell to adopt fate A, whereas the failure to activate *lin-12* above the threshold causes the cell to adopt fate B (Greenwald et al. 1983). Furthermore, inappropriate activation of mammalian *lin-12/Notch* genes have been implicated in oncogenesis (Ellisen et al., 1991; Robbins et al., 1993) and in normal development (e.g. Swiatek et al., 1993). Much of the work in applicants' laboratory is focused on understanding how *lin-12* specifies cell fates. An important component of this endeavor is the identification of genes that influence *lin-12* activity and the identification of potential "downstream" genes.

Applicants identified the *sel-12* gene by screening for suppressors of the "Multivulva" phenotype caused by an allele of *lin-12* that causes constitutive LIN-12 activation. Applicants performed a genetic and molecular characterization of *sel-12*, which established: (1) Reducing or eliminating *sel-12* activity reduces the activity of *lin-12* and of *glp-1*, another member of the *lin-12/Notch* family. In addition,

Summary of the Invention

This invention provides an isolated nucleic acid molecule encoding a SEL-12 protein. This invention further provides an isolated nucleic acid molecule which encodes a mutated SEL-12 protein. This invention also provides an isolated nucleic acid molecule which encodes a mutated SEL-12, wherein the mutated SEL-12 contains at least one of the following: position 115 is a leucine, position 132 is an arginine, position 215 is a glutamic acid, position 229 is a valine, position 254 is a valine, position 255 is a valine, position 371 is a valine, position 387 is tyrosine, position 104 is an isoleucine or position 204 is a valine. This invention further provides different uses of these nucleic acid molecules. This invention also provides different *sel-12* mutants and transgenic animals which carry wild-type or mutated *sel-12*.

Brief Description of the Figures

Figure 1: A. Nucleotide sequence and the deduced amino acid sequence of the *sel-12* cDNA. The first 22 nucleotides, shown in *italics*, correspond to the sequence of the *trans*-spliced leader SL1, a sequence found on the 5' end of many *C. elegans* transcripts 26. Potential membrane-spanning domains are underlined. No potential signal sequence was identified. Analysis of the amino acid sequence using the Kyte-Doolittle algorithm predicts that all nine domains have high enough hydrophobicity values to span a membrane. Three potential glycosylation sites (N-X-T/S) in the region between the seventh and eighth putative transmembrane domains are shown in *italics* at positions 273, 286, and 319 of the amino acid sequence. The locations of the introns are indicated by a caret over the nucleotide preceding the intron. *sel-12* contains seven exons and six introns and spans 2.3 kb of genomic DNA.

B. Schematic representation of the SEL-12 protein and molecular lesions associated with three *sel-12* alleles. Filled rectangles indicate nine hydrophobic regions. Based on the Kyte-Doolittle algorithm, they are potential membrane spanning domains. The fifth hydrophobic region contains only 18 amino acids and the sixth hydrophobic region contains a charged residue; however, these features are conserved in S182, so applicants infer that they are likely to be *bona fide* membrane-spanning domains. The ninth hydrophobic domain is not followed by a basic amino acid and is not conserved in S182 (although the C-terminus of S182 is relatively hydrophobic), so the inference that it is a membrane-spanning domain is more tentative. No potential signal sequence

was identified.

Figure 2: Predicted protein sequence of SEL-12 and its alignment with the predicted protein sequences of S182 and E5-1/STM2. The Pileup program of the GCG-Wisconsin package was used to create this alignment. Amino acids that are identical between SEL-12 and one or more of the other proteins are highlighted in black, and predicted transmembrane domains are overlined. S182 is the predicted protein of a gene associate with early-onset familial Alzheimer's disease (Sherrington et al., 1995). E5-STM2 has also been implicated in early-onset familial Alzheimer's disease (Levy-Lahad et al., 1995a,b; Rogaev et al., 1995). The positions of the ten mutations associated with disease in S182 and E5-1/STM2 (Levy-Lahad et al., 1995b; Rogaev et al., 1995; Sherrington et al., 1995) are indicated (X), and tabulated in Table 1 below. SEL-12 and S182 are 48% identical, SEL-12 and E5-1/STM2 are 51% identical, and S182 and E-51/STM2 are 67% identical (Levy-Lahad et al., 1995b; Rogaev et al., 1995). SPE-4 is the predicated protein of the *spe-4* gene of *C. elegans*, which is required for spermatogenesis (L'Hernault and Arduengo, 1992). SEL-12, S182 and E5-1/STM2 appear to be much more closely related to each other than they are to SPE-4. For example, S182 and SPE-4 are only 22% identical, with several large gaps. Furthermore, several regions that are very highly conserved between SEL-12, S182 and E5-1/STM2 are not conserved in SPE-4, and only one of the ten mutations associated with Alzheimer's disease affects an amino acid that is identical in SPE-4.

Figure 3. Transgenic hermaphrodites expressing a

sel-12::lacZ transgene. Expression is seen in neural and non-neural cells. A. Adult. Large arrow indicates nerve ring; smaller arrows indicate muscle nuclei. B. Adult. Arrows indicate ventral cord nuclei. C. L3 larva. Arrows indicate nuclei of the vulval precursor cells P3.p-P8.p. D. L2 larva. Arrows indicate the nuclei of the somatic gonadal cells Z1.ppp and Z4.aaa. *sel-12* activity has been shown to influence the fates of P3.p-P8.p, and Z1.ppp and Z4.aaa in sensitized genetic backgrounds (11 of the Third Series of Experiments). Compromised neural function associated with reduced activity has not yet been seen in the nerve ring or ventral cord, possibly because an appropriate sensitized genetic background has not been examined. Complete genotype: *smg-1(r861) unc-54(r293); arIs17* [pRF4, pIB1Z17].

Detailed Description of the Inventi n

This invention provides an isolated nucleic acid molecule encoding a SEL-12. This invention further provides an isolated nucleic acid molecule which encodes a mutated SEL-12. This invention also provides an isolated nucleic acid molecule which encodes a mutated SEL-12, wherein the mutated SEL-12 contains at least one of the following: position 115 is a leucine, position 132 is an arginine, position 215 is a glutamic acid, position 229 is a valine, position 254 is a valine, position 255 is a valine, position 371 is a valine, position 387 is tyrosine, position 104 is an isoleucine or position 204 is a valine. In an embodiment, the mutation is generated by in vitro mutagenesis.

In an embodiment, the isolated nucleic acid molecule is a DNA molecule. In a further embodiment, the DNA is a cDNA molecule. In another further embodiment, the DNA is a genomic DNA molecule. In a separate embodiment, the nucleic acid molecule is an isolated RNA molecule.

This invention also provides the above nucleic acid molecule which encodes substantially the same amino acid sequence as shown in Figure 1A.

This invention also provides a nucleic acid molecule of at least 15 nucleotide capable of specifically hybridizing with a unique sequence within the sequence of a nucleic acid molecule described above. In an embodiment, these nucleotide are DNA. In another embodiment, these nucleotide are RNA.

This invention also provides a vector which comprises the above-described isolated nucleic acid molecule. This invention also provides the above-described isolated nucleic acid molecules operatively linked to a promoter of RNA transcription.

In an embodiment, the vector is a plasmid. In an embodiment, the Sel-12 genomic DNA, a MunI/XhoI genomic fragment was cloned into the Bluescript KS* plasmid which was cut with EcoRI and

XhoI. The resulting plasmid is designated as pMX8.

This plasmid, pMX8 was deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. on September 14, 1995 under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganism for the Purposes of Patent Procedure. The pMX8 was accorded with ATCC Accession number 97278.

In another embodiment, a Sel-12 cDNA, an EcoRI cDNA fragment was cloned into the Bluescript KS* plasmid which is cut with EcoRI. The resulting plasmid is designated p1-1E. The plasmid, p1-1E was deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. on September 14, 1995 under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganism for the Purposes of Patent Procedure. The p1-1E was accorded with ATCC Accession number 97279. This plasmid p1-1E containing a frameshift mutation in the 3' end of the coding region of the cDNA. It can be easily corrected to the wild-type sequence as the complete sequence of *Caenorhabditis elegans* has been known.

This invention also provides a host vector system for the production of a polypeptide having the biological activity of a SEL-12 or a mutated SEL-12 which comprises the above-described vector and a suitable host. The suitable hosts include but are not limited to bacterial cells, insect cells, plant and mammalian cells.

This invention also provides purified SEL-12 and mutated SEL-12.

This invention also provides a purified SEL-12 protein or a purified SEL-12 fragment thereof. This invention further provides a purified mutated SEL-12 protein or a purified mutated SEL-12 fragment thereof.

This invention provides a method for production of an antibody

capable of binding to wild-type and/or mutant S182 or E5-1/STM2 comprising: a) administering an amount of the purified protein or fragment of SEL-12 or mutated SEL-12 to a suitable animal effective to produce an antibody against SEL-12 or mutated SEL-12 protein in the animal; and b) testing the produced antibody for capability to bind wild-type and/or mutant S182 or E5-1/STM2.

In an embodiment, the antibody is produced by in vitro immunization. In another embodiment, the antibody is produced by screening a differential phage display library. The produced antibody may be tested by Western blot analysis, immunoprecipitation, staining of cells or tissue sections or in combination of the above.

This invention also provides a method for production of an antibody capable of binding to wild-type and/or mutant S182 or E5-1/STM2 comprising: a) determining conserved regions revealed by alignment of the SEL-12, S182 and E5-1/STM2 protein sequences; b) synthesizing peptides corresponding to the revealed conserved regions; c) administering an amount of the synthesized peptides to a suitable animal effective to produce an antibody against the peptides in the animal; and b) testing the produced antibody for capability to bind wild-type and/or mutant S182 or E5-1/STM2.

In an embodiment, the antibody is produced by in vitro immunization. In another embodiment, the antibody is produced by screening a differential phage display library. The produced antibody may be tested by Western blot analysis, immunoprecipitation, staining of cells or tissue sections or in combination of the above.

This invention provides antibodies produced by above methods. This invention intends to cover other methods of production of antibodies capable of binding to wild-type and/or mutant S182 or E5-1/STM2 using the SEL-12 protein or *sel-12*. This invention also provides monoclonal antibodies capable of binding to wild-type and/or mutant S182 or E5-1/STM2.

This invention also provides antibodies capable of specifically recognizing SEL-12 protein or mutated SEL-12 protein. As used herein the term "specifically recognizing" means that the antibodies are capable of distinquish SEL-12 protein or mutated SEL-12 proteins from other proteins.

This invention also provides transgenic animals which express the above nucleic acid molecules. In an embodiment, the animal is a *Caenorhabditis elegans*. This invention also provides transgenic *Caenorhabditis elegans* animals comprising wild-type or mutant human S182 gene. This invention further provides transgenic *Caenorhabditis elegans* animals comprising wild-type or mutant human STM2/E5-1 gene.

This invention provides the above transgenic *Caenorhabditis elegans* animals, wherein the wild-type or mutant human S182, or wild-type or mutant STM2/E5-1 gene is under the control of *sel-12* or *lin-12* regulatory sequence.

This invention also provides a method for identifying a compound which is capable of ameliorating Alzheimer disease comprising administering effective amount of the compound to the transgenic animals or *sel-12* mutants, the alteration of the conditions of the transgenic animal indicating the compound is capable of ameliorating Alzheimer disease.

This invention also provides a previously unknown compound identified by the above method. This invention provides a pharmaceutical composition comprising an effective amount of the compound identified by the above method and a pharmaceutically acceptable carrier.

Pharmaceutically acceptable carriers are well known to those skilled in the art. Such pharmaceutically acceptable carriers may be aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions

or suspensions, saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, antimicrobials, antioxidants, chelating agents, inert gases and the like.

This invention further provides a method for determining whether a compound might be capable of ameliorating Alzheimer's disease comprising: a) treating *Caenorhabditis elegans* mutants having reduced, increased or altered *sel-12* activity with the compound; and b) determining whether the compound suppresses, enhances or has no effect on the phenotype of the mutant, the suppression or enhancement of the phenotype indicating the compound is capable of ameliorating Alzheimer's disease.

This invention provides a pharmaceutical composition comprising an effective amount of the compound determined by the above method to be capable of ameliorating Alzheimer's disease and a pharmaceutically acceptable carrier.

This invention provides a method for identifying a suppressor of the multivulva phenotype of *lin-12* gain-of-function mutation comprising: a) mutagenizing *lin-12 Caenorhabditis elegans* worms with an effective amount of an appropriate mutagen; b) screening for revertants in the F1, F2 and F3 generations; and c) isolating the screened revertant, thereby identifying a suppressor of the multivulva phenotype of *lin-12*. This invention also provides suppressors identified by the above method.

In an embodiment, this invention provides a *Caenorhabditis elegans* animal having a suppressor, designated *sel-12(ar131)*. This nematode was deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. on September 27, 1995 under the provisions of the Budapest Treaty for the International Recognition of the

Deposit of Microorganism for the Purposes of Patent Procedure. *sel-12(ar131)* was accorded with ATCC Accession number 97293. In another embodiment, this invention provides an animal having a suppressor designated *sel-12(ar133)*.

This invention also provides a method for identifying a mutant *sel-12* gene which reduces *sel-12* function comprising: a) mutagenizing *Caenorhabditis elegans* worms with an effective amount of an appropriate mutagen; b) performing complementation screening of the mutagenized worms to determine if a descendant of a mutagenized worm bears a mutation that fails to complement one of the above-described suppressor for the Egl defect; and c) isolating the individual worm and determining the phenotype of worms carrying the new allele in its homozygous form and *in trans* to a deficiency, thereby identifying a mutant *sel-12* gene which reduces *sel-12* function. In an embodiment, this invention provides the above method which further comprises performing DNA sequence analysis of the identified mutant *sel-12* gene to determine the molecular lesion responsible for the mutation.

This invention also provides mutant *sel-12* genes identified by the above methods. In an embodiment, this invention provides an animal having a mutant *sel-12* gene, designated *sel-12 (ar171)*. This nematode was deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. on September 27, 1995 under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganism for the Purposes of Patent Procedure. *sel-12(ar171)* was accorded with ATCC Accession number 97292.

This invention provides a method for producing extragenic suppressors of a *sel-12* allele comprising: a) mutagenizing *sel-12* mutant hermaphrodites with an effective amount of a mutagen; b) screening for revertants in the F1, F2 and F3 generations; and c) isolating the screened revertant.

This invention also provides a method for producing extragenic

suppressors of a *sel-12(Alz)* mutant comprising: a) mutagenizing *sel-12 (Alz)* hermaphrodites with an effective amount of a mutagen; b) screening for revertants in the F1, F2 and F3 generations; and c) isolating the screened revertant.

Appropriate mutagens which may be used in this invention are well known in the art. In an embodiment, the mutagen is ethyl methanesulfonate.

This invention also provides suppressors produced by the above methods. This invention further provides a method for identification of a suppressor gene comprising performing DNA sequence analysis of the above suppressors to identify the suppressor gene. This invention also provides the identified suppressor gene by the above method.

This invention will be better understood from the examples which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

Experimental Details

First Series of Experiments

Materials and Methods

Applicants genetically mapped *sel-12* to the left of *unc-1 X*: from hermaphrodites of genotype *sel-12(ar131) dpy-3(e27)/unc-1(e538)*, 1/36 Sel non-Dpy and 18/19 Dpy non-Sel recombinants segregated *unc-1*. To clone *sel-12*, applicants used the well correlated genetic and physical maps in the *sel-12* region to identify cosmid clones that potentially carried the *sel-12* gene (ref. 27 and A. Coulson et al., personal communication). Applicants assayed pools and single cosmids for the ability to rescue the Egl defect of *sel-12 (ar131)* hermaphrodites, using the plasmid pRF4 [*rol-6 (su1006)*] as a dominant cotransformation marker (28). Ultimately, applicants found that pSpX4, containing a 3.5 kb*SpeI//Xho I* subclone of C08A12 (Subcloned into KS Bluescript, Stratagene) completely rescue *sel-12(ar131)*. When this subclone was microinjected at a concentration of 10 μg/ml into *sel-12(ar131)* animals, 6/6 lines all demonstrated rescue of the Egl phenotype. When applicants attempted to obtain transgenic lines carrying pSpX4 using a concentration of 50 μg/ml, applicants obtained F1 transformants but no stable lines perhaps indicating some toxicity of this plasmid at higher concentrations. Applicants used this genomic subclone to screen a cDNA library (kindly provided by Bob Barstead) and identified one class of clones of 1.5 kb in size. All subcloning, restriction digests, and library screening were done according to standard techniques (29). Applicants sequenced both strands of the cDNA clone after generating systematic deletions using the Erase-a-base system (Promega®). DNA sequence was performed on double stranded templates using Sequenase (US Biochemical). The cDNA contained both a poly (A) tail and a portion of the spliced leader sequence SL1 (ref. 30), suggesting it was a full length clone. Applicants confirmed the 5′ end of the cDNA by RT-PCR (31). The sequence of this full-length cDNA can be found through GenBank under accession number U35660.

To identify the lesions associated with *sel-12* alleles

applicants used PCR to amplify the *sel-12* genomic fragment from DNA isolated from the *sel-12* mutant strains using the primers DL103 (5'TGTCTGAGTTACTAGTTTTCC 3')(SEQ. ID. 7) and DLG3 (5'GGAATCTGAAGCACCTGTAAGCAT 3')(SEQ. ID. 8). An aliquot of this double-stranded amplification product was used as the template in a subsequent round of PCR using only the primer DL103, to generate a single-stranded template. Exon specific primers were used to determine the entire coding sequence for all three alleles. For each allele, only one alteration in sequence was identified.

Experimental Result and Discussion

The *lin-12(d)* hypermorphic mutation *lin-12(n950)* causes a Multivulva phenotype characterized by the production of ectopic pseudovulvae (3, 4). Applicants screened for non-Multivulva revertants after ethyl methanesulfonate mutagenesis (5) of *lin-12(n950)* hermaphrodites; two recessive suppressors, *ar131* and *ar133*, proved to be alleles of a new gene, *sel-12* (*sel* means suppressor and/or enhancer of *lin-12*). These *sel-12* alleles cause an incompletely penetrant, recessive egg-laying defective (Egl) phenotype in a *lin-12(+)* background. Since *sel-12(ar131)* is viable, fertile and Egl *in trans* to a deficiency (data not shown), applicants also performed a screen for mutations that fail to complement the Egl defect of *sel-12(ar131)*. From a screen of 5900 mutagenized haploid genomes, applicants identified two additional *sel-12* alleles. One allele obtained in this screen, *sel-12(ar171)*, displays a completely penetrant Egl defect as a homozygote and *in trans* to a deficiency, suggesting that *sel-12(ar171)* strongly reduces *sel-12* function. This inference is supported by the molecular analysis described below, which revealed that the *ar171* lesion would result in a truncated protein product.

The Egl phenotype caused by *sel-12* mutations in a *lin-12(+)* background is reminiscent of the Egl phenotype caused by reducing *lin-12* activity (see Table 1 legend). However, a more general involvement of *sel-12* in *lin-12*- and *glp-1*-mediated cell fate decisions becomes apparent when the phenotypes of *lin-12; sel-12* and *glp-1; sel-12* double mutants are analyzed

(Table 1). Applicants examined the genetic interactions of *sel-12* with two *lin-12* hypomorphic mutations, with a *lin-12(d)* hypermorphic mutation, and with a *glp-1* hypomorphic mutation. In all cases, applicants found that reducing *sel-12* activity reduces *lin-12* or *glp-1* activity. These genetic interactions are exemplified by the effects of *sel-12* on two *lin-12*-mediated decisions, the anchor cell/ventral uterine precursor cell (AC/VU) decision and vulval precursor cell (VPC) specification.

The AC/VU decision involves an interaction between two initially equivalent cells of the somatic gonad, Z1.ppp and Z4.aaa. In a given hermaphrodite, Z1.ppp and Z4.aaa interact so that one of these cells becomes the AC while the other becomes a VU (6, 7, 8). When *lin-12* activity is eliminated, both Z1.ppp and Z4.aaa become ACs (the "2 AC defect"), and when LIN-12 is activated, as in *lin-12(d)* mutants, both Z1.ppp and Z4.aaa become VUs (the "0 AC defect") (3,9). Two observations indicate that *sel-12* reduces *lin-12* activity in Z1.ppp and Z4.aaa. First, *sel-12* dramatically enhances the penetrance of the 2 AC defect of *lin-12* hypomorphs (Table 1A). For example, 30% of *lin-12(n676n930)* hermaphrodites have 2 AC (10), whereas essentially all *lin-12(n676n930); sel-12(ar171)* have 2 ACs. Second, *sel-12* partially suppresses the 0 AC defect caused by LIN-12 activation (Table 1B). For example, all *lin-12(n950)* hermaphrodites lack an AC, whereas 10% of *lin-12(n950); sel-12(ar171)* hermaphrodites have an AC.

Table 1.

sel-12(ar171) reduces *lin-12* and *glp-1* activity

A. Enhancement of hypomorphic *lin-12* alleles by *sel-12 (ar171)*

Genotype	% 2ACs	% ventral coelomocytes	fertility	% L1 arrest[k]
wild type[a]	0	0	yes	0
sel-12(ar171)[b]	0	0(0/17)	yes	0(n=233)
lin-12(n676n930)[c]	30g	8(1/12)	yes	9(n=233)
lin-12(n676n930); sel-12(ar171)[d]	95(n=41)	92(12/13)	no	17(n=177)
lin-12(ar170)[e]	16(n=32)	0(0/32)	yes	0(n=209)[i]
lin-12(ar170); sel-12(ar171)[f]	98(n=47)	0(0/47)	yes	0(n=111)
lin-12(0)	100[h]	100[h]	no	10[j]

B. Suppression of a hypermorphic *lin-12* allele by *sel-12(ar171)*

Genotype	number of VPCs adopting a vulval fate/hermaphrodite	% 0 AC
wild type[a]	3	0
lin-12(n950)[l]	6(n=7)	100
sel-12(ar171)[b]	3(n=10)	0(n=108)
lin-12(n950); sel-12(ar171)[m]	2-4(n=8)	89.5(n=57)

C. Enhancement of *glp-1(e2141)* by *sel-12(ar171)*

Genotype	% sterility in both gonad arms	% sterility in one gonad arm
wild type[a]	0	0
glp-1(e2141)[n]	8.5(n=259)	4.0(n=259)
sel-12(ar171)[b]	0	0
glp-1(e2141); sel-12(ar17)[o]	25(n=422)	8.8(n=422)

[a] *C. elegans* var. Bristol strain N2
[b] *sel-12(ar171) unc-1(e538)*
[c] *lin-12(n676n930); unc-1(e538)*
[d] *lin-12(n676n930); sel-12(ar171) unc-1(e538)*
[e] *lin-12(ar170); unc-1(e538)*
[f] *lin-12(ar170); sel-12(ar171) unc-1(538)*
[g] see ref. 10
[h] *lin-12(n137n720)*; see ref. 3
[i] *lin-12(ar170)* [not *unc-1*]
[j] *lin-12(n941)* see ref. 23
[k] some L1 arrested animals were examined for Lag phenotypes, i.e. lack of an anus and rectum, lack of an excretory cell and a twisted nose. These phenotypes were observed for all genotypes where L1 arrested animals were identified.
[l] *lin-12(n950); unc-1(e538)*
[m] *lin-12(n950); sel-12(ar171) unc-1(e538)*
[n] *glp-1(e2141); unc-1(e538)*
[o] *glp-1(e2141; sel-12(ar171) unc-1(e538)*

Table 1. Legend

Most *lin-12*- and *glp-1*-mediated cell fate decisions appear normal in *sel-12(ar171)* mutants. However, the egg-laying defect of *sel-12(ar171)* hermaphrodites resembles the egg-laying defect of *lin-12* hypomorphic mutants (10): *sel-12(ar131)* hermaphrodites leak occasional eggs and larvae, and like *lin-12* hypomorphic mutants, *sel-12* mutants have morphologically normal HSNs, sex muscles and VPC lineages. Egg-laying is particularly sensitive to reduction in *lin-12* activity (10); H. Wilkinson and I.G., unpublished observations). It is therefore possible that both *lin-12* and *sel-12* are required for an as yet unidentified cell fate decision(s) underlying the egg-laying defect. The fact that *sel-12(ar171)* mutants do not display all of the defects associated with loss of *lin-12* function may indicate that *sel-12(ar171)* is not a null allele or *sel-12*

function is partially redundant with the function of another gene.

A. Cell fate transformations were scored at 25° using criteria described in (3) unless otherwise indicated. At 25° *lin-12(n676n930)* behaves like a hypomorph, whereas at 15°C, *lin-12(n676n930)* has mildly elevated *lin-12* activity (10). Since *lin-12(n676n930); sel-12(ar171)* hermaphrodites are sterile at 25°C, applicants shifted fertile *lin-12(n676n930); sel-12(ar171)* hermaphrodites from 15°C to 25°C so that their progeny could be scored for cell fate transformations and other defects. *lin-12(ar170)* behaves like a hypomorph for the AC/VU decision (J. Hubbard and I.G., unpublished observations). In strains containing *lin-12(ar170)*, cell fate transformations were scored in hermaphrodites raised at 20°; other defects were scored in the progeny of hermaphrodites grown at 20° and shifted to 25°.

% 2ACs : In *lin-12(0)* mutants, both Z1.ppp and Z4.aaa become ACs, so *lin-12(0)* hermaphrodites have two ACs; in *lin-12(d)* mutants such as *lin-12(n950)*, both Z1.ppp and Z4.aaa become VUs, so *lin-12(d)* hermaphrodites have 0 ACs. The number of anchor cells was scored in the L3 stage using Nomarski microscopy. For all genotypes, hermaphrodites either had one or two ACs.

ventral coelomocytes: The fates of two pairs of cells, M.d(l/r)pa and M.v(l/r)pa are affected by mutations in *lin-12*. In wild type, the ventral pair of cells gives rise to one sex-myoblast and one body muscle; the dorsal pair gives rise to coelomocytes. In *lin-12(0)* animals, the ventral pair as well as the dorsal pair gives rise to coelomocytes, so that *lin-12(0)* hermaphrodites have extra ventral coelomocytes; in *lin-12(d)* animals, both pairs of cells give rise to sex myoblasts/body muscles. The presence of ventral coelomocytes was scored in the L3 stage. For all genotypes, the absence of ventral coelomocytes suggests that the sex myoblast was specified

normally (see ref. 3).

Fertility: fertility was scored by the appearance of eggs either on the plate or inside the hermaphrodite and the ability to propagate the strain.

L1 arrest: Full viability requires activity of *lin-12* or a related gene, *glp-1*. *lin-12(0) glp-1(0)* double mutants display a fully penetrant L1 arrest phenotype and a Lag phenotype characterized by specific cell fate transformations (23). *lin-12(0)* single mutants display a low penetrance L1 arrest phenotype and a somewhat lower penetrance Lag phenotype (23). Single gravid hermaphrodites were placed on a plate at 25°C. Most of the hermaphrodites were completely egg-laying defective and laid no eggs; some *lin-12(n676n930)* animals released a few eggs or larvae before turning into "bags of worms", in which case the hermaphrodite was transferred after a day. Since *lin-12(n676n930)* animals can grow slowly at 25°C, L1 arrested animals were scored three days after all the eggs had hatched. Arrested L1 animals were spot-checked for the presence of Lag phenotypes using Nomarski microscopy. Some arrested L1 animals of each genotype displayed Lag phenotypes (data not shown).

B. Animals were grown at 20°C. VPC fates were scored by determining the cell lineages of P3.p-P8.p in each animal (Table 2 and data not shown). The number of ACs were scored as described above. For all genotypes, hermaphrodites had either zero or one AC.

C. *glp-1(e2141ts)* is weakly hypomorphic at 20° and essentially wild-type at 15° (24). Strains containing *glp-1(e2141)* were maintained at 15°; fertile adults grown at 15° were placed at 20°, and their progeny grown at 20° were scored for sterility. Other strains were maintained continuously at 20°. *glp-1* activity controls the decision of germline nuclei between mitosis and meiosis (25, 24); L. W. Berry and T. Schedl, personal communication). GLP-1

is thought to be the receptor for the inductive signal from the distal tip cells of the somatic gonad that promotes germline mitosis (and/or inhibits meiosis) (7). When *glp-1* activity is eliminated, germline nuclei enter meiosis (25). Hermaphrodites of each genotype were scored for sterility in one or both gonad arms in the dissecting microscope. Several sterile or half-sterile individuals were examined by Nomarski microscopy, and sterile gonad arms were found to have the characteristic Glp phenotype (data not shown).

Each of the six VPCs, P3.p-P8.p, has the potential to adopt one of two vulval fates, termed "1°" and "2°", or a non-vulval fate, termed "3°" (11, 12). Normally, P5.p, P6.p, and P7.p adopt vulval fates, in a 2°-1°-2° pattern (13). This pattern is the outcome of the integration of two signalling inputs: a *let-60* Ras-mediated inductive signal from the AC induces vulval fates, and a *lin-12*-mediated lateral signal between VPCs prevents adjacent VPCs from adopting the 1° fate (reviewed in ref. 14). The *let-60* Ras-mediated inductive signal may cause expression or activation of the lateral signal (15, 16), which activates LIN-12 to cause a VPC to adopt the 2° fate (3, 17, 18).

Reducing *sel-12* activity reduces *lin-12* activity in lateral signalling that specifies the 2° fate of VPCs. First, *sel-12* reduces the effect of activated LIN-12 in the VPCs: all VPCs adopt the 2° fate in *lin-12(n950)* hermaphrodites, but only half of the VPCs adopt the 2° fate in *lin-12(n950); sel-12(ar171)* hermaphrodites (Table 1b, Table 2). Second, *sel-12* reduces lateral signalling that occurs upon activation of *let-60* Ras. Applicants analyzed VPC lineages (data not shown) in *let-60(n1046)* hermaphrodites, in which Ras has been activated by a codon 13 mutation (19, 20), and in *let-60(n1046); sel-12(ar171)* hermaphrodites. Lateral signalling appears to occur normally in *let-60(n1046)* hermaphrodites, since adjacent VPCs do not adopt the 1° fate (0/20 pairs of induced VPCs). In contrast, adjacent VPCs sometimes adopt the 1° fate in *let-60(n1046);*

sel-12(ar171) hermaphrodites (4/18 pairs), implying that reducing the activity of *sel-12* reduces lateral signalling. Finally, some VPCs adopt the 2° fate in *lin-12(n676n930)* hermaphrodites (10). In contrast, VPCs do not adopt the 2° fate in *lin-12(n676n930); sel-12(ar171)* double mutants (data not shown), although applicants have not tested whether this effect is due to the presence of a second AC.

Table 2.

sel-12(ar171) plays a role in the receiving cells

Genotype	Expression of 2° fate/total P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	% VPCs adopting a 2° fate hermaphrodite
lin-12(n950)	7/7	7/7	7/7	7/7	7/7	7/7	100
lin-12(n950); sel-12(ar171)	0/8	1/8	4/8*	8/8	6/8	2/8**	52
lin-12(n-950)	X	11/11	X	X	X	X	100
lin-12(n950); sel-12(ar171)	X	3/10	X	X	X	X	30

Table 2. Legend

X=cell killed by a laser microbeam. Numbers in each column correspond to the proportion of times a given VPC was observed to adopt the 2° fate (criteria as in ref. 18). All VPCs that did not undergo 2° fates underwent 3°, or non-vulval fates, with three exceptions: *= in 1/8 animals examined, P5.p underwent a hybrid (2°/3°) lineage; **= in 2/8 animals examined, P8.p underwent a hybrid (2°/3°) lineage. Animals were maintained at 20°C. Early L2 hermaphrodites (as judged by the size of the gonad) were chosen for laser ablation studies. The fates of the VPCs have not been determined at this time; the VPCs become determined many hours later, in the L3 stage (Sternberg and Horvitz, 1986). P3.p, and P5.p-P8.p were destroyed with a laser microbeam; the success of this operation was verified 2-3 hours later. The following day, the operated animals were mounted for Nomarski microscopy so that the cell lineage of P4.p could be observed directly. In both operated

and unoperated animals, vulval fates were scored by directly observing the cell lineage of each VPC. The operated animals were observed until the early L4 stage, to ensure that no divisions were missed.

The genetic interactions of *sel-12* with *lin-12* imply a function for *sel-12* in signalling and/or receiving cells during lateral specification. Applicants have tested whether *sel-12* functions in the receiving end of *lin-12*-mediated cell-cell interactions by performing cell ablation experiments (Table 2). Applicants reasoned that, if all VPCs but one were ablated with a laser microbeam, the fate of the isolated VPC would reflect its intrinsic level of *lin-12* activity in the absence of lateral signal. Thus, in *lin-12(n950)* hermaphrodites, an isolated VPC adopts the 2° fate (Table 2), suggesting that it has a high level of ligand-independent activation of LIN-12 in the VPCs (9). If *sel-12* were to function in one VPC to lower *lin-12* activity in another, then in *lin-12(n950); sel-12(ar171)* hermaphrodites, an isolated VPC should also adopt the 2° fate. However, if *sel-12* were to function within a VPC to lower its *lin-12* activity, then in *lin-12(n950); sel-12(ar171)* hermaphrodites, an isolated VPC should instead adopt the 3° fate. Applicants observed that in *lin-12(n950); sel-12(ar171)* hermaphrodites, an isolated P4.p often adopts the 3° fate (Table 2), implying that *sel-12* functions within a VPC to lower *lin-12* activity.

Applicants cloned *sel-12* by transformation rescue (Fig. 1 legend), and determined the nucleotide sequence of a full-length cDNA (Genbank Accession number U35660). The predicted SEL-12 protein contains multiple potential transmembrane domains (Fig. 1B), consistent with SEL-12 function as a receptor, ligand, channel, or membrane structural protein. The SEL-12 protein is evolutionarily conserved. Database searches revealed a high degree of similarity to a sequence of a partial cDNA from human brain present on clone T03796 and a low degree of similarity to SPE-4, a protein required for *C. elegans*

spermatogenesis (21). In addition, SEL-12 is highly similar to S182, which, when mutant, has been implicated in familial early-onset Alzheimer's Disease (22). T03796 has recently been shown to correspond to the E5-1/STM2 gene, which has also been implicated in early onset familial Alzheimer's disease (Levy-Lahad et al., 1995a,b; Rogaev et al., 1995). The predicted protein sequences of SEL-12, ES-1/STM2, SPE-4, and S182 are aligned in Fig. 2.

lin-12/Notch genes specify many different cell fate decisions in *C. elegans* and Drosophila, and in both organisms some of these decisions are critical for neurogenesis. The genetic analysis described here indicates that *sel-12* facilitates *lin-12*-mediated reception of intercellular signals. *sel-12* might be directly involved in *lin-12*-mediated reception, functioning for example as a co-receptor or as a downstream effector that is activated upon LIN-12 activation. Alternatively, *sel-12* may be involved in a more general cellular process such as receptor localization or recycling and hence influence *lin-12* activity indirectly. Although the remarkable conservation of *sel-12* and S182 does not provide any immediate indication of the function of S182 in the Alzheimer's disease process, it is striking that 4 of the 5 mutations found in affected individuals alter amino acids that are identical in SEL-12 and S182 (see Fig. 2). The powerful tools of classical and molecular genetic studies in *C. elegans*, including the ability to identify extragenic suppressor and to generate transgenic lines containing engineered genes, can now be brought to bear on fundamental issues of SEL-12/S182 structure and function.

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Second Series of Experiments

BACKGROUND AND SIGNIFICANCE

Alzheimer's disease is a devastating and common disease of the central nervous system, and studies of familial forms have identified a number of loci that are implicated in the development of the disease. Two loci, S182 (*AD3*) (Sherrington et al., 1995) and *STM2* (Levy-Lahad et al., 1995a,b), which is also known as *E5-1* (Rogaev et al., 1995), have recently been found to be associated with the development of early onset familial Alzheimer's disease. These loci encode related proteins with multiple transmembrane domains.

The *C. elegans* model described here is based on the finding that the *sel-12* gene encodes a protein that is highly similar to S182 and STM2 (Levitan and Greenwald, 1995; see Fig. 1). For example, SEL-12 and S182 are 48% identical over 460 amino acids. The remarkable conservation of the SEL-12 and S182 predicted protein structure suggests that their functions are likely to be conserved as well. Furthermore, it is striking that seven of the eight changes in S182 that are associated with early-onset familial Alzheimer's disease (Rogaev et al., 1995; Sherrington et al., 1995; see Fig. 1) alter amino acids that are identical in SEL-12, and that the eighth alters an amino acid that has been changed very conservatively during evolution, and two out of two changes in STM2/E5-1 that are associated with Alzheimer's disease (Levy-Lahad et al., 1995b; Rogaev et al., 1995) affect amino acids that are identical in SEL-12.

Applicants hope to bring the powerful tools of classical and molecular genetic studies in *C. elegans* to bear on fundamental issues of SEL-12/S182/STM2 structure and function. Thus far, proteins similar to LIN-12/Notch and SEL-12/S182/STM2 have not been described in single-celled organisms (for example, >95% of the yeast genome has been sequenced and has not yielded any similar proteins), so *C. elegans* may be the simplest practical system for studying these issues in vivo.

PRELIMINARY STUDIES

s 1-12. Applicants identified *sel-12* [*sel* =*suppressor/enhancer of lin-12*] by screening for suppressors of the "Multivulva" phenotype caused by an allele of *lin-12* that causes constitutive LIN-12 activation. Applicants performed a genetic and molecular characterization of *sel-12* (Levitan and Greenwald, 1995), which established: (1) Reducing or eliminating *sel-12* activity reduces the activity of *lin-12* and of *glp-1*, another member of the *lin-12/Notch* family. In addition, reducing or eliminating *sel-12* activity causes an egg-laying defective (Egl) phenotype. Applicants do not know if the Egl phenotype is a direct consequence of reducing *lin-12* activity (Sundaram and Greenwald, 1993a) or an independent effect of reducing *sel-12* activity. (2) *sel-12* and *lin-12* can functionally interact within the same cell. (3) *sel-12* is predicted to encode a protein with multiple transmembrane domains that is highly similar to S182 and STM2, which have been implicated in early-onset familial Alzheimer's disease (Levy-Lahad et al., 1995a, b; Rogaev et al., 1995; Sherrington et al., 1995). The presence of multiple transmembrane domains is consistent with SEL-12 function as a receptor, ligand, channel or membrane structural protein.

The fact that the only striking phenotype caused by *sel-12(ar171)* is a defect in egg-laying may reflect the fact that egg-laying is particularly sensitive to reduction in *lin-12* activity (Sundaram and Greenwald, 1993a; H. Wilkinson and I.G., unpublished observations). The egg-laying defect may reflect an as yet unidentified cell fate decision(s), or alternatively may also be viewed as a late-onset behavioral phenotype. However, the fact that *sel-12(ar171)* mutants do not display all of the defects associated with loss of *lin-12* function may indicate that *sel-12(ar171)* is not a null allele, despite the severe truncation in protein product it is expected to cause; alternatively, *sel-12* function may be partially redundant with the function of another gene.

Applicants identified a genomic fragment capable of complementing *sel-12* alleles (Levitan and Greenwald, 1995).

Some of the experiments described in this invention require the ability to express reporter genes or altered *sel-12* genes appropriately. An expression method developed in applicants' laboratory will enable these experiments to be performed. (1) Applicants have developed a vector that expresses inserted cDNAs under the control of *lin-12* regulatory sequences (pLEX; Struhl et al., 1993). The applicants have found that construct containing a *sel-12* cDNA in the pLEX vector is capable of rescuing *sel-12* mutants. (2) Applicants have developed an analogous vector, p1B7, that should express inserted cDNAs under the control of *sel-12* regulatory sequences. p1B7 is based on a genomic fragment that is capable of rescuing *sel-12* mutants (Levitan and Greenwald, 1995): a unique BamH1 site was inserted at +1 into a genomic fragment capable of complementing a mutant allele, thereby destroying the first codon of the gene. The expression vector contains 3.5 kb of 5' flanking region (2.5 kb more than the original rescuing fragment of Levitan and Greenwald, 1995) and 0.5 kb of 3' flanking region.

These vectors are used as follows (Wilkinson et al., 1994; Fitzgerald and Greenwald, 1995; Wilkinson and Greenwald, 1995). A cDNA containing its own start and stop codons, but lacking a polyadenylation signal, is inserted into the vector. The resulting transcript is predicted to contain an unusually long 3' untranslated region (UTR). These aberrant 3' UTRs are generally destabilizing, leading to very low levels of detectable expression. However, this problem can be overcome by placing the transgenes in a *smg* mutant background, which stablizes mRNAs with long 3' untranslated regions (Pulak and Anderson, 1993). The recent identification of a temperature-sensitive *smg*-7 mutation (B. Cali and P. Anderson, personal communication) enables transgenic lines to be generated at the permissive temperature (15°), where *smg*-7(ts) has nearly wild-type activity, and shifted to the restrictive temperature (25°) for the analysis of mutant phenotypes (K. Fitzgerald, personal communication).

lin-12. *lin-12* is the archetype of the "*lin-12/Notch* gene family" of putative transmembrane receptor proteins that is

found throughout the animal kingdom (reviewed in Greenwald and Rubin, 1992; Artavanis-Tsakonas et al., 1995). Members of this family are transmembrane proteins with repeated epidermal growth factor (EGF)-like motifs and LIN-12/Notch repeat motifs in their extracellular domains, and "cdc10/SWI6" motifs (also termed "ankyrin repeats") in the intracellular domains. In *C. elegans* and Drosophila, *lin-12/Notch* family members were first defined genetically, by mutations that alter cell fate decisions that involve cell-cell interactions during development (reviewed in Greenwald and Rubin, 1992). In vertebrates, *lin-12/Notch* genes were identified either by cross-hybridization with *Notch* probes, or, more revealingly, by oncogenic mutations: mutation of *int-3* by mouse mammary tumor virus is associated with the development of breast cancer in mice (Gallahan and Callahan, 1987; Robbins et al., 1992) and mutation of *TAN-1* is associated with T cell leukemias in people (Ellisen et al., 1991; Robbins et al., 1992).

The nature of the relationship between *lin-12* and *sel-12* is uncertain. *lin-12/Notch* genes specify many different cell fate decisions in *C. elegans* and Drosophila, and in both organisms some of these decisions are critical for neurogenesis. As described above, the initial genetic analysis indicated that *sel-12* facilitates *lin-12*-mediated reception of intercellular signals (Levitan and Greenwald, 1995). *sel-12* might be directly involved in *lin-12*-mediated reception, functioning for example as a co-receptor or as a downstream effector that is activated upon LIN-12 activation. Alternatively, *sel-12* may be involved in a more general cellular process such as receptor localization or recycling and hence influence *lin-12* activity indirectly. The powerful tools of classical and molecular genetic studies in *C. elegans*, including the ability to identify extragenic suppressors and to generate transgenic lines containing engineered genes, can now be brought to bear on fundamental issues of SEL-12/S182/STM2 structure and function.

RESEARCH DESIGN AND METHODS

I. Basic characterization of *sel-12*.

A. Additional basic characterization of *sel-12*. There are several lines of experimentation that, along with previous work (Levitan and Greenwald, 1995), will constitute the basic characterization of *sel-12*.

(1) Null phenotype. Although *sel-12(ar171)* is predicted to encode a protein that is truncated by half, it is conceivable that this portion of the protein retains some activity and that *sel-12(ar171)* is not a true null allele [*sel-12(ar171)* mutants have normal mRNA levels]. Null alleles will be used to reveal the requirement for gene activity, for gene dosage studies, and as a background into which engineered *sel-12* mutations can be introduced. Applicants will therefore isolate additional *sel-12* alleles by complementation screening as described in Levitan and Greenwald (1995), with the goal of identifying an internal deletion of *sel-12* or an allele associated with a stop codon early in the gene. If alleles with early stops or internal deletions cause a more severe phenotype than *sel-12(ar171)*, applicants will analyze the phenotype in detail. Alleles with other properties may also be obtained from the screen + may be useful for other experiments, such as drug testing.

(2) Expression pattern. Using the expression vector p1B7 applicants have engineered a *sel-12::lacZ* reporter gene. The *lacZ* gene used contains a nuclear localization signal (Fire et al., 1990), which facilitates the identification of individual cells. A developmental profile of expression will be determined. Preliminary results indicate that *sel-12::lacZ* is more broadly expressed than *lin-12::lacZ* (Wilkinson and Greenwald, 1995), including much expression in the nervous system.

(3) Behavioral defects. Besides the Egl defect of hermaphrodites, there may be other behavioral defects. For example, preliminary results suggest that *sel-12(ar171)* males display behavioral abnormalities that affect mating efficiency. Applicants will examine this potential defect further using mating assays (Hodgkin, 1983; Liu and Sternberg, 1994). The *sel-12::lacZ* expression pattern may provide clues for behaviors

that may be affected in *sel-12* mutants.

(4) SEL-12 antibodies. Applicants will use standard methods (Harlow and Lane, 1988) to generate antibodies to SEL-12. The antibodies will be useful for examining protein localization: the localization of wild-type and mutant SEL-12 proteins in otherwise wild-type backgrounds and in suppressor mutant backgrounds.

(5) Identification of *C. elegans* genes that are highly related to SEL-12. One possible reason that the phenotype of *sel-12(ar171)* is of relatively limited severity is that *sel-12* is partially functionally redundant with another gene or genes. Functional redundancy might be reflected in sequence similarity. The *C. elegans spe-4* gene (L'Hernault and Arduengo, 1992) is weakly related to *sel-12* (see Fig.1) and in collaboration with Steve L'Hernault (Emory University), applicants will express a *spe-4* cDNA under the control of *sel-12* or *lin-12* regulatory sequences, to see if SPE-4 can replace SEL-12. Applicants will also examine the phenotype of *spe-4; sel-12* double mutants to see if the double mutant has a more sever phenotype than either single mutant.

If more closely related genes exist, applicants can easily identify them by periodically searching the database of the *C. elegans* sequencing project, which is currently 25%complete, and is expected to be fully completed by 1998 (R. Waterston et al., personal communication). It may also be possible to identify *sel-12* related genes by low-stringency hybridization (Sambrook et al., 1989) and/or screening an expression library with SEL-12 antibodies (Harlow and Lane, 1988). If any method identifies genes that are related to *sel-12*, applicants will express them under the control of *sel-12* or *lin-12* regulatory sequences to see if they can functionally replace *sel-12*. If so, then applicant will attempt to generate null alleles of the *sel-12*-related gene, using a Tc1 transposon-based excision method (Rushforth et al., 1993; Zwaal et al., 1993; Greenwald et al., 1994), unless better gene "knock-out" technology becomes available. The phenotype of null mutants will be

examined alone, and in combination with *sel-12*(null).

It is also possible that genes similar to *sel-12* will be revealed by the analysis of other genes identified by reverting alleles of *lin-12* (Sundaram and Greenwald, 1993b; J. Thomas, F. Tax, E. Ferguson and H.R. Horvitz, personal communication; D. Levitan and I.Greenwald., unpublished observations).

B. Functional equivalence of S182, STM2 and SEL-12. There is high degree of similarity between SEL-12, S182, and STM2, which suggests they have similar biochemical functions and properties. The best test of this hypothesis would be to demonstrate that S182 and STM2 can substitute for SEL-12. Applicants will place the human cDNAs under the control of *sel-12* regulatory sequences, using the p1B7 expression vector and will assess the ability of S182 or STM2 to replace SEL-12 in *C. elegans*.

II. Engineered *sel-12* transgenes ["*sel-12(Alz)*"] containing alterations associated with early-onset familial Alzheimer's disease

The experiments in this section of the proposal are designed to help understand the consequences of mutation of S182 and STM2 for protein function. Mutations that alter the SEL-12 protein so that they resemble mutant proteins associated with familial early-onset Alzheimer's disease will be created. Because genetic analysis in *C. elegans* has revealed the phenotypic consequences of reducing *sel-12* activity as well as the phenotypic consequences of both reduced and elevated *lin-12* activity, genetic analysis of phenotypes associated with *sel-12(Alz)* mutations will reveal the effect of S182 and STM2 mutations on S182 and STM2 function.

A. Generation of transgenic *C. elegans* lines. Applicants will create engineered *sel-12* transgenes containing alterations associated with early-onset familial Alzheimer's disease in people. Applicants will engineer the changes using standard PCR-based strategies in a clone of *sel-12* genomic DNA. These

clones will be microinjected into *lin-12(+); sel-12(+) C. elegans* (either the wild-type strain N2 or usefully marked derivatives) to establish transgenic lines (Fire, 1986; Mello et al., 1991), which will be analyzed for mutant phenotypes and for interactions with *lin-12*. The *rol-6(su1004)* gene (Mello et al., 1991) will be used as a cotransformation marker; other cloned genes may be used as cotransformation markers to facilitate phenotypic analysis, which can be difficult in Roller mutants, if necessary. Several different concentrations of injected DNA will be tried.

Table 3.

Human gene	Mutation	SEL-12 residue
S182	M146L	M115
	H163R	H132
	A246E	V215
	A260V	A229
	A285V	A254
	L286V	L255
	L382V	L371
	C410Y	C387
STM2	N141I	N104
	M239V	M202

Table 3. Mutations associated with the development of Alzheimer's disease (Levy-Lahad et al., 1995; Rogaev et al., 1995; Sherrington et al., 1995), and the corresponding amino acid in SEL-12 (see also Figure 1). Note that nine of ten mutations in S182 or STM2 affect amino acids that are identical in SEL-12. The tenth, S182 A246E, causes a dramatic change in a residue that is conservatively different between S182 and SEL-12.

If the *sel-12(Alz)* mutations cause dominant lethal or sterile phenotypes that prevent the establishment of transgenic lines, applicants will use an alternative strategy to achieve conditional or more limited expression. The engineered mutations will be incorporated into a *sel-12* cDNA, which can be cloned into a *sel-12* expression vector applicants have made (see "Background and Preliminary Studies"): in this vector, the ATG of the cloned *sel-12(+)* gene has been replaced by a BamH1 linker, so that cDNAs can be cloned into the unique BamH1 site and expressed under the control of *sel-12* regulatory sequences. Efficient expression should be obtained in a *smg*

mutant background, so that transgenic arrays may be generated in a *smg(+)* background and crossed into a *smg* background for analysis, or generated in a *smg-7(ts)* background at the permissive temperature (15°) and analyzed at the restrictive temperature (25°). The temperature-sensitive *smg-7* mutant will be particularly useful, since transgenic worms may be shifted at different times during development, and the effects on different cell fate decisions examined.

Applicants can also clone the mutant *sel-12* cDNAs into a *lin-12* expression vector (Struhl et al., 1993), which has a more restricted pattern of expression (defined by Wilkinson et al., 1994; Wilkinson and Greenwald, 1995) and hence may be less deleterious. Although heat shock promoter-based vectors are available, in applicants' experience they have not been reliably effective for studies of *lin-12*-mediated cell fate decisions, probably because of tissue-specificity of the heat shock promoters (see Stringham, Fire). However, they may be useful for examining the consequences of altered *sel-12* coding regions in other tissues, or for ectopic expression experiments.

Applicants can also perform analogous experiments using mutated human *S182* or *E5-1/STM2* cDNAs cloned into p1B7 or pLEX.

Applicants will create integrated lines for phenotypic analysis. In *C. elegans*, the microinjection technique used to establish transgenic lines generally results in lines containing extrachromosomal arrays of injected DNAs. Such extrachromosomal arrays may be integrated by irradiation (Hedgecock and Herman, 1995), so that arrays become inserted randomly into the genome. Such lines generally have more reproducible expression from the transgenes, and avoid complications for phenotypic analysis introduced by the potential for somatic mosaicism of extrachromosomal arrays.

B. Phenotypic analysis of transgenic lines containing *sel-12(Alz)* genes. Integrated lines carrying *sel-12(Alz)* genes will be analyzed for viability and fertility. They will also

be examined for the Egl phenotype associated with reduced *sel-12* activity (Levitan and Greenwald, 1995), and other phenotypes that may be revealed by the analysis described in section I of this proposal. They will also be analyzed for phenotypes associated with reduced *lin-12* activity (such as 2 anchor cells, no 2° vulval precursor cell lineages, ventral coelomocytes/missing sex muscles; Greenwald et al., 1983; Sundaram and Greenwald, 1993a) or elevated *lin-12* activity (such as no anchor cell, ectopic 2° vulval precursor cell lineages, extra sex muscles/no dorsal coelomocytes; Greenwald et al., 1983), and reduced *glp-1* activity (such as germline proliferation defect, missing anterior pharynx or extra pharyngeal cells; Austin and Kimble, 1987; Priess et al., 1987; Bowerman et al., 1994; Mello et al., 1994) or elevated *glp-1* activity (Fitzgerald and Greenwald, 1995; tumorous germ line; L. W. Berry and T. Schedl, personal communication).

If it is necessary to use a conditional expression system to generate the lines, transgenic animals will be examined after a shift from the permissive to the restrictive temperature at different times during development.

If antibodies to SEL-12 are available, the localization of wild-type and mutant SEL-12 proteins will be examined by examining stained whole-mounts by confocal microscopy and possibly by immunoelectron microscopy.

C. Genetic analysis of *sel-12(Alz)* genes. The S182 and STM2 mutations associated with early onset Alzheimer's disease in people are dominant. The most likely possibility is that altered gene activity underlies this dominance, since ten different mutations in S182 and STM2 are missense mutations in conserved amino acids. Dominant mutations may cause a mutant protein to have elevated activity, decreased activity, or aberrant activity. Genetic tests can be used to distinguish these possibilities, and are particularly valuable when biochemical function is not known or when biochemical assays are difficult to execute on mutant proteins. Thus, the ability to assess the genetic properties of the *sel-12(Alz)* transgenes

in *C. elegans*, where rigorous genetic tests to determine the consequences of mutation on gene activity are possible, may be very valuable for understanding the effect of the mutations on Alzheimer's disease loci in people.

If *sel-12(Alz)* mutations cause dominant phenotypes in *C. elegans* (i.e., phenotypes in a *sel-12(+)* background), applicants will examine them by adapting classical gene-dosage tests (Muller, 1932) for hypermorphic (elevated), neomorphic (novel) or antimorphic (dominant-negative) activity. Two approaches will be used. First, established arrays carrying *sel-12(Alz)* genes will be crossed into *sel-12(ar171)* mutants, and into *sel-12(+)* hermaphrodites carrying a duplication of *sel-12(+)*. Second, additional arrays will be established by coinjection of *sel-12(Alz)* with *sel-12(+)* genes. If a *sel-12(Alz)* mutation is a hypermorph, then the severity of the mutant phenotype should increase as additional doses of *sel-12(+)* are added. If a *sel-12(Alz)* mutation is a neomorph, then the severity of the mutant phenotype should be essentially unchanged as additional doses of *sel-12(+)* are added. If a *sel-12(Alz)* mutation is an antimorph, then the severity of the mutant phenotype should decrease as additional doses of *sel-12(+)* are added.

If *sel-12(Alz)* does not cause a phenotype in a *sel-12(+)* background, the *sel-12* activity of the transgenes will be assessed by placing the transgenes into a *sel-12(ar171)* or *sel-12(null)* background. If the *sel-12(Alz)* transgenes do not have rescuing activity, then applicants will not be able to draw any rigorous conclusions.

III. Identification and characterization of extragenic suppressors of *sel-12(ar171)* and *sel-12(Alz)*

Extragenic suppressor mutations may identify new genes that are involved in SEL-12/S182/STM2-mediated processes. Even if suppressor mutations identify genes that were defined previously, they will reveal a functional connection with *sel-12/S182/STM2*. Genetic and molecular characterization of these

"suppressor genes" in *C. elegans* will reveal the nature of their interactions with *sel-12* and *lin-12*. Furthermore, if suppressor mutations, or other alleles of suppressor genes that can be subsequently generated (such as null alleles), have highly-penetrant, easily scored phenotypes, they too can be reverted to identify additional genes that may be involved in *sel-12* function. In this way, a network of interacting genes can be identified, and the normal function, as well as the aberrant function in mutants, can be elucidated.

A potential outcome of the suppressor analysis is an insight into the biochemistry of SEL-12/S182/STM2-mediated processes. The best outcome will be if one of the suppressor genes has a known biochemical activity (based on sequence analysis). This information will be combined with the results of genetic analysis suggesting the nature of the interaction of the suppressor mutations with *sel-12*, and will potentially be useful for the design and testing of therapeutic agents in both *C. elegans* and mammalian models, and ultimately for people. A second important reason is that human homologs of the suppressor genes themselves may be useful diagnostic reagents. For example, such cloned genes might be used to analyze human pedigrees to reveal the underlying defects in other inherited forms of Alzheimer's disease (and will possibly have some use for sporadic forms as well).

A. Reversion of *sel-12(ar171)*. *sel-12(ar171)* causes a highly penetrant Egl phenotype. Applicants will generate Egl$^+$ revertants by mutagenizing *sel-12(ar171)* hermaphrodites with ethyl methanesulfonate (EMS) (Brenner, 1974) and screening for Egl$^+$ (normal egg-laying) revertants in the F_1, F_2 and F_3 generations. This procedure will enable the identification of dominant, recessive and maternal effect suppressor mutations.

Applicants performed a pilot mutagenesis, which indicated that this procedure will yield suppressor mutations: applicants identified two suppressor mutations, including a dominant suppressor that maps near *dpy-10 II* (D. Brousseau, personal communication), in a region of the genome that has been well

characterized genetically (e.g., Sigurdson et al., 1984) and sequenced (R. Waterston et al., personal communication). The suppressor mutations appeared to arise at low frequency, suggesting that they may be specific alterations and not null alleles, but applicants did not perform careful quantitation in their pilot experiment. Future mutageneses for suppressor mutations will be performed quantitatively (see e.g., Greenwald and Horvitz, 1980).

B. Reversion of *sel-12(Alz)* mutants. If *sel-12(Alz)* mutations cause a highly penetrant phenotype (such as lethality, sterility, or egg-laying defect), applicants will mutagenize integrated lines and look for revertants.

C. Analysis of suppressor ("*sup*") mutations.

(1) Basic genetic analysis. This analysis will include:

(a) Mapping and complementation tests. Applicants will determine if the *sup* mutation is recessive or dominant, precisely map the suppressor mutations and perform complementation testing with candidate genes in the region, and perform *inter se* complementation testing among recessive *sup* mutations mapping in the same region.

(b) Phenotypic analysis. The phenotype of *sup* mutations in a *sel-12(+)* background, and in combination with *lin-12* activated (Greenwald et al., 1983; Greenwald and Seydoux, 1990; Struhl et al., 1993), *lin-12* hypomorphic (Sundaram and Greenwald, 1993a), and *lin-12(null)* (Greenwald et al., 1983) alleles will be examined. The localization of wild-type and mutant SEL-12 proteins will be examined by examining stained whole-mounts by confocal microscopy and possibly by immunoelectron microscopy.

(c) Gene dosage studies. Genetic studies will be used to illuminate the effect of the *sup* mutation on *sup* gene activity. For a recessive suppressor, the relative suppression of *sup/Df* and *sup/sup* will be compared; these genotypes will also be examined for additional phenotypes. The genotype *sup/sup/+*

will also be examined if an appropriate duplication is available, since it is possible that the *sup* mutations are recessive gain-of-function and require two copies to suppress *sel-12* mutations.

For a dominant suppressor, the relative suppression of *sup/Df*, *sup/+* and *sup/+/+* will be compared, by examining the ability to suppress *sel-12* mutations and by analyzing any associated mutant phenotypes. The rationale is the same as described above: if a *sup* mutation is a hypermorph, then the suppression ability (and/or an associated phenotype) should increase as additional doses of *sup-?(+)* are added; if *sup* is a neomorph, then the suppression ability (and/or phenotype) should be essentially unchanged as additional doses of *sup-?(+)* are added; and if a *sup* mutation is an antimorph, then the suppression ability (and/or mutant phenotype) should decrease as additional doses of *sel-12(+)* are added.

(d) Null phenotype of *sup* genes. If *sup* mutations are not null alleles, then applicants will perform screens for null mutations. For example, if the *sup* mutations are recessive partial loss-of-function mutations and are viable and fertile in *trans* to a deficiency, then applicants can screen for *sup/*; sel-12* hermaphrodites that are suppressed (where * = mutagenized chromosome) (see e.g. Greenwald and Horvitz, 1980). If the *sup* mutations are dominant, then applicants can screen for loss of dominant suppressor activity in *sup */+; sel-12* hermaphrodites (see e.g. Greenwald and Horvitz, 1982). The null phenotype of *sup* loci may reveal the normal role of *sup* genes.

(2) Molecular analysis. The first phase of molecular analysis involves the molecular cloning and DNA sequence analysis of suppressor genes. Transposon tagging (Greenwald, 1985; Moerman et al., 1986), or transformation screening of clones from the well-correlated genetic and physical maps (Coulson et al., 1988 and personal communication) can be used to clone genes in *C. elegans*. The details of such strategies require the completion of the genetic analysis of the suppressor

mutations. A general overview of such strategies is given below.

Transposon-tagging: Suppressor genes may be cloned by screening for transposon-associated alleles, using the same strategies as can be used for identifying null alleles described above. Potential transposon-associated alleles can be screened by Southern blotting, using transposon probes (e.g., Greenwald, 1985; Moerman et al., 1986), or cosmids in the region provided by the genome project.

Transformation screening: Suppressor genes defined by loss-of-function or antimorphic (dominant-negative) mutations may be cloned by transformation "antisuppression": cloned cosmids provided by the genome project may be used to establish transgenic arrays that complement *sup* mutations, thereby reversing their ability to suppress mutations in *sel-12*. This strategy may also be adapted to clone suppressor genes defined by gain-of-function hypermorphic or neomorphic mutations. After a *sup* mutation has been mapped to a small region of the physical map, cosmids from the region can be used to probe a Southern blot of DNA made from the *sup* mutant, in the hopes of identifying an altered restriction fragment associated with the *sup* mutation. If an alteration is not detected, then a modified transformation screening approach may be used. A library can be made from a *sup* mutant, and DNA from the region can be identified by probing with mapped cDNAs from the region provided by the genome project. The potential *sup* containing cosmids can be verified by restriction mapping or DNA fingerprinting (Coulson et al., 1986), and used for transformation experiments based on their dominant suppressor activity.

Identification of other genes whose activities are influenced by *sel-12*. Applicants are testing the genetic interaction of *sel-12* alleles with mutations in other secreted or transmembrane proteins by constructing and analyzing double mutants. This information may reveal other pathways that involve *sel-12* activity, and may suggest other human diseases

for which sel-12 is relevant.

Identification of other genes involved in *sel-12*-mediated processes by the yeast two-hybrid system. Applicants will apply the yeast two-hybrid system to screen a cDNA library for potential interacting proteins and to screen directly for interaction with LIN-12 and GLP-1. The two-hybrid screen, originally developed by Fields and Song (1989), is a powerful strategy for identifying potential interacting proteins. the screen relies on the ability of GAL4 to activate transcription of a reporter gene containing GAL4 upstream activation sequences. GAL4 has a DNA binding domain (GBD) and an activation domain (GAD). Normally, the two domains are present in the same polypeptide; if they are separated, GAL4 activity is abolished. However, if the separated domains are joined to protein sequences that interact with each other, the two domains are brought together, and GAL4 activity is restored. Thus, a yeast strain containing a "bait" fused to the GBD is transformed with a library containing potential GAD fusions, and a selection or screen for reconstituted GAL-4 activity is used to identify candidates.

The virtue of conducting such a screen in *C. elegans* is the potential for genetic analysis of candidate genes, since in the absence of a functional analysis it is possible that physical interactions revealed by the two-hybrid method are not meaningful in vivo. Mutations that reduce or eliminate the activity of the candidate gene will be analyzed in *C. elegans*. If the candidate clone maps to a genetically well-characterized region, applicants will try transformation rescue of the extant mutations. Alternatively, null alleles will be identified using PCR-based screens (Rushforth et al., 1993; Zwaal et al., 1993; Greenstein et al., 1994). The consequences of elevating candidate gene activity will be examined by creating high copy number transgenic lines or by overexpressing the candidate gene in wild-type and mutant backgrounds. Any candidate genes that appear to be involved in SEL-12- mediated processes by genetic analysis can be used in the same way the suppressor *"sup"* genes described above could be used.

The use of *sel-12* mutants for scr ening for compounds that may ameliorate Alzheimer's disease, and possibly other diseases caused by affecting the activity of members of the SEL-12/S182/STM2 family. *sel-12* mutants generated by standard genetic and transgenic methods may be use for drug testing. This approach is potentially beneficial for two reasons. First using C. *elegans*, the applicants can analyze the effect of drugs on *sel-12* activity even though the biochemical function of *sel-12* is not known, based on the suppression or enhancement of *sel-12* mutant phenotypes (i.e, egg-laying defect and other phenotypes that will be identified, or the effects of altering *sel-12* activity on *lin-12* activity). For example, the proportion of egg-laying competent *sel-12(ar131)* or *sel-12(ar171)* mutant hermaphrodites may be compared when the mutant worms are cultured in the presence of candidate compounds; an increase in the proportion of egg-laying competent worms in the presence of compound would indicate that *sel-12* activity is increased or bypassed. *sel-12* mutants may also be transiently treated with candidate compounds. If the *sel-12(Alz)* mutations have additional or different phenotypic consequences, transgenic lines containing *sel-12(Alz)* transgenes may also be used to screen for the effect of compound on *sel-12(Alz)* activity. Second, *C. elegans* is easy and inexpensive to cultivate. Thus, a preliminary screening of the effect of compounds on *sel-12* mutants may help to set priorities for drug testing in mammalian system, thereby reducing the expense and shortening the amount of time it takes to identify potential therapeutic agents.

Since *sel-12* mutations affect *lin-12* activity, and mammalian homologues of *lin-12* have been implicated in oncogenesis, it is possible that the identification of compounds that influence *sel-12* activity will have implications for cancer, and possibly other human diseases.

Implications of suppressor genes for drug testing. Suppressor genes defined genetically, and candidates defined using the yeast two-hybrid system, encoding proteins of known biochemical

function will be useful for targeted drug design or the development of diagnostic tests for Alzheimer's disease or other diseases associated with alteration of members of the SEL-12/S182/STM2 family. For example, if a suppressor gene encodes a protein with an enzymatic activity, competitive or noncompetitive inhibitors of the enzyme might be effective drugs.

Suppressor genes encoding proteins of unknown biochemical function will also be useful for drug development. For example, the use of ribozymes based on suppressor genes, or the delivery via liposomes of vectors expressing suppressor genes, are potential therapeutic applications. The genetic analysis in *C. elegans* will provide a guide as to the nature of suppressor mutations. For example, a mutation that suppresses a *sel-12(Alz)* mutation that increases the activity of the suppressor gene would suggest the second strategy.

Implications of suppressor genes for diagnostic tests. The genetically-defined suppressor genes or candidate genes obtained using the yeast-two hybrid system will be used to identify human homologues. The cloned human homologues will be used to analyze pedigrees to see if mutations of the suppressor loci are associated with the development of Alzheimer's disease or other diseases. For example, the E5-1 gene was identified by using a cloned gene for pedigree analysis (Rogaev et al., 1995).

Suppressor genes may also be used as the basis for diagnostic tests. For example, mutations in suppressor genes implicated in Alzheimer's disease will be detected at the DNA level by Southern blotting or PCR/sequencing analysis, or at protein level, by Western blotting, immunoprecipitation or staining of cells or tissues.

Antibodies for diagnosis. Antisera to SEL-12 may cross-react with S182 and/or E5-1/STM2. Furthermore, peptides designed on the recognition of highly conserved regions, revealed by alignment of the predicted protein sequences of SEL-12, S182,

and E5-1/STM2, or of SEL-12, S182, E5-1/STM2, and SPE-4 (see Fig. 2), may be useful as diagnostic reagents. The conserved regions may reveal salient characteristics of a family of proteins, two of which have already been implicated in early-onset Alzheimer's disease. Such antisera could also be used to identify other members of the family, by screening expression libraries (Harlow and Lane, 1988).

References of the Second Series of Experiments

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Third Series of Experiments
Assessment of normal and mutant human presenilin function in *C. el gans*

Applicants provide evidence that normal human presenilins can substitute for *C. elegans* SEL-12 protein in functional assays *in vivo*. In addition, six familial Alzheimer's disease-linked mutant human presenilins were tested and found to have reduced ability to rescue the *sel-12* mutant phenotype, suggesting that they have lower than normal presenilin activity. A human presenilin 1 deletion variant that fails to be proteolytically processed and a mutant SEL-12 protein that lacks the carboxy terminus display considerable activity in this assay, suggesting that neither presenilin proteolysis nor the carboxy terminus is absolutely required for normal presenilin function. Applicants also show that *sel-12* is expressed in most neural and non-neural cell types in all developmental stages. The reduced activity of mutant presenilins together with as yet unknown gain-of-function properties may be a contributing factor in the development of Alzheimer's disease.

Genetic linkage studies have identified a number of genetic loci associated with familial Alzheimer's disease (1). Mutations in two genes, encoding the presenilins PS1 and PS2, are dominant and fully penetrant (1, 2, 3, 4, 5). PS1 and PS2 are related multipass transmembrane proteins that are about 67% identical in amino acid sequence. The presenilins are ubiquitously expressed (4, 5), and found in conjunction with intracellular membranes (6).

The normal function of presenilins, and the mechanism by which mutant presenilins cause Alzheimer's disease, are not yet known. The fact that more than thirty dominant, fully penetrant mutations in PS1 and PS2 are all missense mutations has suggested that Alzheimer's disease is associated with a gain-of-function activity of mutant proteins, although it remains formally possible that they partially lower activity of a dose-sensitive gene. Indeed, mutations may also have more than one effect on gene activity, and may have both

gain-of-function and loss-of-function characteristics. Classical studies have indicated that gain-of-function mutations in principle fall into one of three classes: hypermorphic mutations, which elevate gene activity; antimorphic mutations, which reduce wild-type gene activity in *trans* (this category includes dominant-negative mutations); and neomorphic mutations, which create a novel activity (7). However, at the biochemical level, even the novel activity resulting from neomorphic mutations is related to the normal mechanism of gene function. For example, neomorphic mutations in the Drosophila *awd* gene appear to alter the substrate specificity of nucleoside diphosphate kinase as well as reduce activity for its normal substrate (8), and mutations that cause familial amyotropic lateral sclerosis affect different activities of the normal protein, increasing the level of peroxidase activity (9) while in some cases reducing superoxide dismutase activity (10). Thus, an understanding of the normal function of presenilins as well as the nature of the dominant mutations is crucial to elucidating the role of mutant presenilins in Alzheimer's disease.

Genetic studies in simple organisms offer a powerful approach to understanding the role of presenilins. A *C. elegans* gene, *sel-12*, encodes a protein that displays about 50% amino acid sequence identity to PS1 and PS2 (11). *sel-12* was identified by reverting a phenotype caused by constitutive activation of LIN-12, a member of the LIN-12/Notch family of receptors [*sel* =*suppressor/enhancer of lin-12*]. Genetic analysis established that reducing or eliminating *sel-12* activity reduces the activity of *lin-12*, and causes an egg-laying defective (Egl) phenotype. The Egl phenotype may be a direct consequence of reducing *lin-12* activity (12) or an independent effect of reducing *sel-12* activity. In this paper, applicants provide evidence that SEL-12 and the presenilins are functional homologs, and that studies in *C. elegans* will be directly applicable to issues of presenilin structure and function in humans.

MATERIALS AND METHODS

General m thods and mutations used. Methods for handling and culturing *C. elegans* have been described (13). The wild-type parent for all strains used was *C. elegans* var. Bristol strain N2 (13). *sel-12(ar131)* is described in ref. 11. All strains containing pLEX-based plasmids (see below) contained the *smg-1(r861)* and *unc-54 (r293)* mutations (14). *smg-1* mutations stabilize mRNAs with long 3' untranslated regions (15), and *unc-54(r293)* is suppressed by *smg-1(r861)* (14). **pLEX-based constructs.** The pLEX vector has been described previously (16). It contains a 15.1 kb genomic region encompassing the *lin-12* gene, in which the normal translational start ATG was destroyed and replaced with a Not I site. cDNAs containing stop codons but lacking polyadenylation signals are inserted into the Not I site, and are efficiently expressed in a *smg-1* background. The following cDNAs were inserted into pLEX for this study.

***sel-12*:** The *sel-12* cDNA is described in ref. 11 and, as described below, results in efficient rescue of a *sel-12* mutant. Applicants note here that the *C. elegans* genome project has sequenced through the *sel-12* region (R. Waterston et al., personal communication). By comparing the genomic sequence with that of the available *sel-12* cDNA, applicants discovered that the cDNA has a frameshift mutation, beginning at codon 413, probably introduced by reverse transcription. This frameshift results in the substitution of 31 amino acids C-terminal to the frameshift mutation by 49 amino acids.

PS1: Full-length human PS1 cDNA and cDNA encoding the PS1 A246E substitution were generated by RT-PCR of cytoplasmic RNA isolated from skin fibroblasts of a patient harboring the A246E mutation (NIA Cell Repository #AG06848B) using a sense primer, hAD3-ATG-Kpn (GGGGTACCATGACAGAGTTACCTGCAC), and antisense primer, hAD3-R-3'UTR (CCGGGATCCATGGGATTCTAACCGC). PCR products were digested with Asp718 and BamHI and ~1.4 kB hPS1 cDNAs were gel purified and ligated to Bluescript KS+ vector (Stratagene, La Jolla, CA.) previously digested with Asp718 and BamHI, to

generate phPS1 and phPS1A246E. The cDNAs were sequenced in their entirety using a Sequenase kit (U.S. Biochemical Corp., Cleveland, OH).

To generate human PS1 cDNA encoding the M146L, H163R, L286V or C410Y substitutions (5), applicants used a four-way PCR strategy with two primer pairs and full-length PS1 cDNA as template. The inserts and junctions were sequenced using Sequenase (U.S. Biochemical Corp. (Cleveland, OH).

For M146L, primer pairs were hAD3-M146LF (GTCATTGTTGTCCTGACTATCCTCCTG)/hAD3-R284 (GAGGAGTAAATGAGAGCTGG) and hAD3-M146LR (CAGGAGGATAGTCAGGACAACAATGAC)/hAD3-237F (CAGGTGGTGGAGCAAGATG). PCR products from each reaction were gel purified, combined and subject to a second round of PCR with primers hAD3-237F and hAD3-R284. The resulting product was digested with KasI and PflMI and an ~300 bp gel purified fragment was ligated to KasI/PflM1-digested phPS1 to generate phPS1M146L. For H163R, primer pairs were hAD3-H163RF (CTAGGTCATCCGTGCCTGGC)/hAD3-R284 and hAD3-H163RR (GCCAGGCACGGATGACCTAG)/hAD3-237F. PCR products from each reaction were gel purified, combined and subject to a second round of PCR with primers hAD3-237F and hAD3-R284. The resulting products were digested with KasI and PflMI and a gel-purified ~300 bp fragment was ligated to KasI/PflM1-digested phPS1 to generate phPS1H163R.

For L286V, primer pairs were hAD3-L286VF (CGCTTTTTCCAGCTGTCATTTACTCC)/ hAD3-RL-GST (CCGGAATTCTCAGGTTGTGTTCCAGTC) and hAD3-L286VR (GGAGTAAATGACAGCTGGAAAAAGCG)/ hAD3-F146 (GGATCCATTGTTGTCATGACTATC). PCR products from each reaction were gel purified, combined and subject to a second round of PCR with primers hAD3-F146 and hAD3-RL-GST. The resulting products were digested with PflMI and BbsI and a gel purified ~480 bp fragment was ligated to PflMI/BbsI-digested phPS1 to generate phPS1L286V.

For C410Y, primer pairs were hAD3-C410YF

(CAACCATAGCCTATTTCGTAGCC)/ LRT7 (GCCAGTGAATTGTAATACGACTCACTATAGGGC) and hAD3-C410YR (GGCTACGAAATAGGCTATGGTTG)/hAD3-243S (CCGGAATTCTGAATGGACTGCGTG). PCR products from each reaction were gel purified, combined and subject to a second round of PCR with primers hAD3-243S and LRT7. The resulting products were digested with BbsI and BamHI and an ~300 bp fragment was gel purified and ligated to BbsI/BamHI-digested phPS1 to generate phPS1C410Y.

The strategy for generating cDNA encoding hPS1 lacking exon 9 (amino acids 290-319) was described previously (17).

PS2: Full-length cDNA encoding human PS2 was generated by RT-PCR of total human brain RNA using a sense primer, huAD4-ATGF (CCGGTACCAAGTGTTCGTGGTGCTTCC) and antisense primer, hAD4-stopR (CCGTCTAGACCTCAGATGTAGAGCTGATG). PCR products were digested with Asp718 and XbaI and ~1.4 kB hPS2 cDNA were gel isolated and ligated to a vector fragment from expression plasmid pCB6 (17) previously digested with Asp718 and XbaI to generate phPS2. The insert was sequenced in its entirety using a Sequenase kit (U.S. Biochemical Corp., Cleveland, OH).

Transgenic lines and rescue assays. Transgenic lines were established by microinjection of plasmid mixtures into the hermaphrodite germline to create extrachromosomal arrays (18). By accepted convention, "Ex" is used to represent extrachromosomal arrays, and "Is" to represent integrated arrays (which can be generated from extrachromosomal arrays; see below).

pLEX and derivatives were injected at 20 μg/ml, 2 μg/ml or other concentrations (data not shown) into recipient strains of genotype *smg-1(r861) unc-54(r293); sel-12(ar131)* or *smg-1(r861) unc-54(r293)*. pRF4, a plasmid containing the cloned dominant *rol-6(su1006)* gene (18) was used as a cotransformation marker and coinjected at a concentration of 100 μg/ml. F1 Roller progeny were picked, and F2 Roller progeny used to establish lines.

To assess rescue of *sel-12(ar131)*, approximately 40 L4 Rol progeny from at least three independent lines generated in a *smg-1(r861) unc-54(r293); sel-12(ar131)* background were picked individually and scored daily for the ability to lay eggs. Applicants note here that rescue assays were performed using *sel-12(ar131)*, a strong partial loss-of-function allele of *sel-12*, because the strongest existing *sel-12* mutation, *sel-12(ar171)*, is somewhat suppressed by *smg-1* (data not shown). *sel-12(ar131)* displays variable penetrance (see Table 4) and expressivity. About 10% of *sel-12(ar131)* hermaphrodites have normal egg-laying, while 90% of hermaphrodites bloat with retained eggs; some of these bloated hermaphrodites never lay eggs, whereas others lay eggs. However, the proportion of hermaphrodites that lay eggs normally appears to be reduced by the pLEX vector and/or the *rol-6* cotransformation marker (see Table 4). Applicants scored hermaphrodites as "Egl$^+$" only if they displayed robust egg-laying characteristic of wild-type hermaphrodites after two days as adults. However, applicants note that a greater proportion of hermaphrodites containing human wild-type and mutant presenilins displayed improved egg-laying after one day compared to control hermaphrodites (data not shown), indicating that the criterion of normal egg-laying after two days underestimates rescuing activity. The pLEX vector causes a low level of sterility, and sterile hermaphrodites were not scored.

Table 4

transgene*	line	Egl*/total (%)'
none	-	3/44 (6.8)
pLEX	1	1/71 (1.4)
	2	0/36 (0)
	3	1/40 (2.5)
SEL-12'	1	36/39 (92.3)
	2	38/40 (95.0)
	3	40/40 (100)
PS1	1	30/44 (68.1)
	2	33/40 (83.0)
	3	32/40 (80.0)
PS2	1	26/39 (67.0)
	2	33/40 (83.0)
	3	32/40 (80.0)
PS1 M146L	1	4/39 (10.3)
	2	6/37 (16.2)
	3	2/29 (6.9)
PS1 H163R	1	12/38 (31.6)
	2	7/38 (18.4)
	3	23/38 (60.5)
PS1 A286E	1	4/36 (11.1)
	2	5/39 (12.8)
	3	3/39 (7.7)
PS1 L266V	1	11/38 (28.9)
	2	6/38 (15.8)
	3	9/38 (23.7)
PS1 C410Y	1	7/36 (19.4)
	2	2/35 (5.7)
	3	7/38 (18.4)
PS1 ΔE9	1	26/39 (66.7)
	2	28/38 (73.7)
	3	17/27 (63.0)

Rescue of the *sel-12* egg-laying defective (Egl) and abnormal vulva phenotypes by normal and mutant human presenilins. The data is shown for transgenic lines generated by injecting the construct being tested at a concentration of 20 μg/ml. See Methods for details about generating and scoring transgenic lines.

* Most PS1 mutations that cause Alzheimer's disease affect amino acids that are identical in SEL-12. The amino termini of PS1, PS2 and SEL-12 are not well conserved and are of different lengths. Therefore, for the mutations used here, the amino acid corresponding to M146 in PS1 is M115 in SEL-12; PS1 H163 is SEL-12 H132; PS1 A246 is SEL-12 V216; PS1 L286 is SEL-12 L255; PS1 C410 is SEL-12 C384. The ΔE9 mutation inhibits cleavage of PS1 (17); applicants note that SEL-12 is cleaved in a comparable position (Li and Greenwald, submitted).

' Egl* signifies robust egg-laying characteristic of wild-type hermaphrodites after two days as adults. This criterion is the most stringent applicants could apply, and underestimates the degree of rescuing activity (see Materials and Methods).

' Note that the *sel-12* cDNA used (11) has a frameshift mutation,

beginning at codon 413, resulting in the substitution of 31 amino acids C-terminal to the frameshift mutation by 49 amino acids (see Materials and Methods). See Materials and Methods for details about the human presenilin cDNAs.

Transgenic lines and ß-galactosidase staining. pIB1Z17 [*sel-12::lacZ*] was made as follows: A unique *Bam*HI site was inserted using the polymerase chain reaction at the second amino acid of a *sel-12* rescuing genomic fragment containing 2.8 kb of 5' flanking region. A *lacZ* gene encoding a ß-galactosidase protein containing a nuclear localization signal was excised from plasmid pPD16.43 (19) and inserted in frame into the *Bam*HI site to generate the plasmid pIB1Z17. The predicted transcript contains an abnormally long 3' untranslated region, consisting of the *sel-12* coding and 3' untranslated region, and is expected to be stabilized in a *smg-1* background (15). pIB1Z17 was injected at a concentration of 10 µg/ml into *smg-1 unc-54* hermaphrodites. 9 independent lines containing extrachromosomal arrays were established. 4 independent attached lines were generated (using the method of C. Kari, A. Fire and R.K. Herman, personal communication) from one of the extrachromosomal arrays. All integrated and 7 of the 9 extrachromosomal arrays displayed staining; all staining lines had similar expression patterns, but some lines displayed more variability in intensity or penetrance of staining. The analysis described in this paper was performed using the attached array *arIs17*.

Mixed stage populations were grown at 25°, fixed using an acetone fixation protocol (20) and stained for ß-galactosidase activity overnight at room temperature. Stained nuclei were identified based on their size, shape and position (21,22). Counterstaining with 4,6-diamidino-2 phenylindole (DAPI) allowed visualization of all nuclei in the animal by fluorescence microscopy, facilitating the unambiguous identification of stained nuclei. Pictures of the staining pattern were taken at 1000X using TMAX400(Kodak) film.

RESULTS

A presenilin functional assay. There are currently no biochemical assays for presenilins, so there has been no direct assay for the effects of mutations on presenilin function. The high level of similarity between SEL-12, PS1 and PS2 suggested that the ability to rescue the distinctive egg-laying defective (Egl) phenotype caused by mutations that reduce or eliminate *sel-12* activity (11) could serve as an assay for presenilin function. The pLEX vector (16), which places inserted cDNAs under the control of *lin-12* regulatory sequences, can direct sufficient expression of a full-length *sel-12* cDNA (11; see Materials and Methods) to rescue the *sel-12(ar131)* Egl phenotype (Table 4). Applicants describe below how applicants have used this assay to evaluate the activity of normal and mutant human presenilins.

Rescue is assessed in transgenic lines, which are created by the microinjection of plasmid DNA into the hermaphrodite germline. This procedure generates extrachromosomal arrays, and there is some inherent variability in expression from different arrays, in part due to different numbers of copies of plasmid incorporated into the array (18). However, variability can be controlled for by examining multiple independent lines for each construct. Furthermore, arrays generated at the same concentration of injected DNA are likely to have comparable numbers of plasmid copies and therefore comparable levels of transgene expression (18). In all of the experiments described below, applicants have examined three independent lines for each construct, and compare the results for lines generated at the same concentration of injected DNA.

Rescue of a *sel-12* mutant by wild-type PS1 and PS2. Applicants have assessed the ability of wild-type human PS1 or PS2 cDNAs to rescue the Egl defect of *sel-12(ar131)* hermaphrodites (Table 4). Applicants found that the human proteins can efficiently substitute for SEL-12 in this assay, despite the vast evolutionary distance between nematodes and humans. The human proteins seem to be slightly less efficient than the *C. elegans* protein, but this small difference might in principle result

from inefficient translation of human presenilin RNA due to the different codon usage between *C. elegans* and humans, so that less presenilin protein may be produced even if a comparable level of mRNA is expressed from the extrachromosomal arrays. The dramatic increase in *sel-12* activity when PS1 or PS2 is expressed using *lin-12* regulatory sequences, even at a relatively low concentration of injected DNA (Table 5), suggests that the human proteins are substituting for *C. elegans* SEL-12. An alternative interpretation is that the human protein functions in this assay by stabilizing the mutant endogenous SEL-12(ar131) protein. However, this interpretation seems less likely in view of the efficient rescue; furthermore, a corrective interaction of this sort would imply that a SEL-12 and PS1 or PS2 complex is functional, which in itself would be evidence for functional similarity of the *C. elegans* and human proteins.

Activity of PS1 point mutants. Applicants expressed five different human mutant PS1 proteins, each containing a single amino acid alteration that causes Alzheimer's disease, and found that most displayed reduced ability to rescue *sel-12(ar131)* relative to wild-type PS1 (Table 4). These data suggest that the mutations that cause Alzheimer's disease may reduce but not eliminate normal presenilin activity. The variable loss of extrachromosomal arrays confounds any determination of steady-state protein levels, so applicants do not know if the apparently lower activity of mutant presenilins results from reduced protein stability or reduced function.

Activity of PS1 ΔE9. PS1 is subject to endoproteolysis in vivo, and the PS1 ΔE9 mutant fails to be cleaved (17). Applicants have found that the human mutant PS1 ΔE9 retains a high level of activity, when arrays are formed at the concentration of 20 μg/ml of injected DNA (Table 4). Since arrays generated at a concentration of 20 μg/ml of injected DNA are likely to contain many plasmid copies, which might mask a small difference in relative activity of PS1 and PS1 ΔE9, applicants generated arrays at the concentration of 2 μg/ml of injected DNA. At this concentration of injected DNA, the

number of copies of plasmid present in the arrays should be reduced roughly tenfold (Mello et al., 1991). At this lower concentration, PS1 ΔE9 has reduced ability to rescue *sel-12(ar131)* as compared to wild-type PS1 (Table 5), suggesting that PS1 ΔE9, like the PS1 missense mutations, has reduced activity.

Table 5

transgene	line	Egl*/total (%)*
pLEX	1	1/35 (2.9)
	2	0/38 (0)
SEL-12†	1	38/40 (95.0)
	2	40/40 (100)
	3	8/20 (40.0)
PS1	1	8/31 (25.8)
	2	36/41 (87.8)
	3	34/37 (92.0)
	4	33/40 (91.9)
	5	34/40 (85.0)
PS1 ΔE9	1	6/37 (16.2)
	2	5/39 (12.8)
	3	5/37 (13.5)
	4	14/41 (34.1)
	5	1/40 (2.5)

Rescue of the *sel-12* Egl phenotype by PS1 and PS1 ΔE9 expressed from arrays formed at a concentration of 2 μg/ml. At 2 μg/ml of injected DNA, expression from arrays or representation of the plasmid in the arrays may be reduced, accounting for the reduced activity of SEL-12 (transgenic line 3) and PS1 (transgenic line 1) compared to arrays generated at 20 μg/ml (Table 4).

* Egl*, see Table 4 legend and Materials and Methods.

† see Table 4 legend and Materials and Methods for comments about the *sel-12* cDNA used.

Examination of PS1 mutant transgenes in a *sel-12(+)* background. In an attempt to reveal gain-of-function activity, applicants assayed the ability of transgenes encoding mutant presenilins to cause phenotypes in a *sel-12(+)* background. Applicants saw no evidence for gain-of-function activity in this assay, as measured by the failure to obtain highly penetrant Egl or vulval abnormalities associated with abnormal *sel-12* or *lin-12* activity (data not shown). However, intrinsic limitations of the pLEX expression system (see Materials and Methods) may have

masked moderate changes in *sel-12* or *lin-12* activity, so a definitive assessment of the gain-of-function activity of mutant presenilins in *C. elegans* will not be possible until other expression systems or strategies are developed.

***sel-12* is widely expressed in neural and non-neural cells.** Applicants have examined the expression pattern of transgenic lines carrying a *sel-12::lacZ* reporter gene (see Materials and Methods). Using this reporter gene, applicants have found that *sel-12*, like human presenilins (4, 5), is widely expressed in neural as well as non-neural cells (Fig. 3). Staining was seen in most cell types at all developmental stages from embryo to adult, with the notable exception of the intestine.

DISCUSSION

Sequence analysis revealed that SEL-12 is similar to human presenilins (11). Here, applicants have provided experimental evidence that SEL-12 is a bona fide presenilin, since it may be functionally replaced by either of the two human presenilins. Applicants have also shown that *sel-12* is widely expressed in most neural and non-neural tissues of developing animals and adults. Furthermore, SEL-12 and PS1 also appear to have similar membrane topology (Doan et al., submitted; Li and Greenwald, submitted). These striking parallels between *C. elegans* and human presenilins suggest that studies of SEL-12 in *C. elegans* will bear directly on fundamental issues of presenilin structure and function. In the absence of any description of proteins similar to presenilins in single-celled organisms, including Saccharomyces cerevisiae, it appears that *C. elegans* is the simplest practical system for studying issues relevant to the biology of presenilins *in vivo*.

Since PS1 and PS2 appear to be similar in their ability to substitute for SEL-12, they may also have overlapping functions in mammals. As a consequence, studies of normal and mutant PS1 proteins should be directly applicable to PS2, and vice versa. Furthermore, since PS1 and PS2 have broad and overlapping expression patterns (4, 5), the phenotype of

mutants homozygous for null alleles of individual mouse presenilin genes may be less severe than the phenotype of double mutants, since there may be functional redundancy where the expression patterns overlap.

The rescue experiments also provide an indication that two regions of the presenilins are not essential for normal function. First, a SEL-12 protein lacking the last 31 amino acids is highly functional (see Table 4), suggesting that the C terminus is dispensable for SEL-12 function. Second, the PS1 ΔE9 protein, which 30 amino acids and fails to be proteolytically cleaved (17), retains considerable activity, suggesting that neither the deleted region nor cleavage is a prerequisite for presenilin activity. Applicants note that the rescue experiments do not address the possibility that the various mutations applicants tested have gain-of-function activity. Although the nature of the hypothetical gain-of-function activity of mutant presenilins is not clear, the mutant presenilins appear to increase the extracellular concentration of Aß1-42(43) (ref. 23; Borchelt et al., submitted), and hence may cause Alzheimer's disease by fostering Aß deposition.

By expressing human genes in *C. elegans*, applicants have obtained evidence that six different presenilin mutations that cause early-onset Alzheimer's disease lower normal presenilin activity. Hypomorphic characteristics were manifested as reduced ability to rescue a *C. elegans* mutant defective in *sel-12* presenilin function. In the absence of any other assays for normal presenilin function, this information may be useful in considering the pathogenesis of Alzheimer's disease, and the development of mammalian models for the disease. It is possible that reduced presenilin activity may contribute to the development of Alzheimer's disease, either directly or in conjunction with an as yet unknown gain-of-function activity associated with mutant presenilins.

Gain-of-function activity of *s 1-12(Alz)* transgenes

The applicants have modified the *C. elegans sel-12* gene to encode mutant proteins corresponding to PS1 mutants that cause Alzheimer's disease in people. Transgenic *C. elegans* lines containing these *sel-12(Alz)* genes have a novel gain-of-function activity (manifested as an egg-laying constitutive (Egl^c) phenotype), which may be mechanistically related to a gain-of-function activity that is presumed to underlie the development of Alzheimer's disease. The penetrance of the Egl^c phenotype is enhanced in a *sel-12(ar171)* background. An Egl^c phenotype has been known to be associated with stimulation of a G protein coupled serotonergic neural pathway in *C. elegans* (Segalat et al., 1995; Mendel et al., 1995; Koelle and Horvitz, 1996). The applicants are currently exploring the effects of *sel-12(Alz)* mutations on other neural signalling pathways that involve G protein coupled 7 transmembrane domain receptors, and neural signalling pathways that may involve other kinds of signal transduction pathway.

sel-12 mutant transgene	line	Egl^c/Egl^+ (%)
+	1	0/37 (0)
	2	1/38 (2.6)
	3	0/38 (0)
H132R	1	2/38 (5.3)
	2	5/36 (13.9)
	3	2/39 (5.1)
V216E	1	2/31 (6.5)
G363A	1	11/31(35.5)
	2	13/40(32.5)
	3	16/40(40.0)

Data shown are for transgenes in a *sel-12(ar171)* genetic background.

It may be that drugs that reduce serotonergic signalling or other signalling pathways that the applicants will test will suppress *sel-12(Alz)* gain-of-function phenotypes, thereby

suggesting potential prophylactic or therapeutic treatments, particularly if these signalling pathways or related pathways are shown to be affected in Alzheimer's disease. It may also be that the effect of drugs that reduce the gain-of-function activity of mutant presenilins will be potentiated by drugs that increase the normal activity of presenilins.

spr* Genes: Suppressors of *sel-12(ar171)

sel-12(ar171) hermaphrodites are egg-laying defective (Egl). The applicants have identified more than fifty extragenic suppressors of the Egl defect of *sel-12(ar171)* after EMS mutagenesis. The applicants have thus far assigned seven of the semidominant suppressor mutations to four new genes, named *spr-1* through *spr-4* [*spr* stands for *s*uppressor of *p*resenilin]. Two recessive suppressors probably define two additional *spr* genes. The remaining mutations are currently being analyzed and will be assigned to genes based on map position, genetic properties, and for recessive mutations, by complementation tests.

Gene dosage studies suggest that *spr-1V* mutations are hypermorphic, and that excess copies of the wild-type locus suppress *sel-12(ar171)*. The applicants are currently performing equivalent gene dosage studies with *spr-2 II*, which has been mapped to a 0.25 map unit interval corresponding to about 200 kb, and with *spr-3 III*. Meanwhile, assuming that the *spr-2* mutation is hypermorphic and that excess copies of the wild-type locus will suppress *sel-12(ar171)*, the applicants have embarked on cloning *spr-2* by injecting pools of cosmid clones from the *spr-2* region into *sel-12(ar171)*, and preliminary data suggest that this strategy will be successful.

The identification of suppressor mutations is a classical genetic tool used to identify other components of biochemical pathways. Extragenic suppressor mutations may identify new genes that are involved in presenilin-mediated processes, or reveal a functional connection between a previously known gene and presenilin function. Genetic and molecular

characterization of these "suppressor genes" in *C. elegans* will reveal the nature of their interactions with *sel-12* and *lin-12*. This analysis is directly relevant to Alzheimer's disease because the biochemical function of the presenilins is not known, so that a potential outcome of analyzing a suppressor gene would be an insight into the biochemistry of presenilin-mediated processes. If the suppressor gene has a known biochemical activity (based on sequence analysis), then, combined with the results of genetic analysis, the information will potentially be useful for the design and testing of therapeutic agents in both *C. elegans* and mammalian models, and ultimately for people. Furthermore, human homologs of the suppressor genes themselves may be useful diagnostic reagents, perhaps for the analysis of other inherited forms of Alzheimer's disease or for sporadic forms.

Topology and structure/function studies

The applicants have obtained evidence that SEL-12 presenilin contains 8 transmembrane domains (Li and Greenwald, submitted), and that certain regions of presenilins are dispensable for normal presenilin activity (Levitan et al., submitted). The applicants are continuing to do structure/function studies, by engineering mutant *sel-12* transgenes and assessing them in vivo in transgenic *C. elegans* lines for the ability to rescue defects associated with reducing *sel-12* activity and for gain-of-function activity.

Further structure/function studies in C. elegans may clarify the functions of domains of presenilin and be useful in conjunction with ultrastructural studies for rational drug design.

Gene and allele specificity studies

The applicants have been making double mutants between *sel-12(ar171)* and mutations in other secreted or transmembrane proteins. Thus far, a genetic interaction has been seen with a mutation in a TGF-ß receptor gene, *daf-1*. This result

suggests that *sel-12* may interact with genes other than *lin-12* and *glp-1*.

genotype	%Daf
daf-1(m213)	13%
daf-1(m213); sel-12(ar171)	98%

Interactions of this sort may enable the design of other suppressor/enhancer screens.

Other *C. elegans* presenilin genes

The applicants regularly search the *C. elegans* genomic sequence database for sequences related to *sel-12*. Recently, a predicted protein encoded by a sequence present on cosmid C18E3 was found to have significant similarity to SEL-12. The applicants will test any potentially related sequences for the ability to complement *sel-12(ar131)* as described in Levitan et al. (submitted). Any sequences that behave like SEL-12/presenilins by this functional assay will be studied further.

Other *C. elegans* presenilins can be studied in the same way as *sel-12* in order to gain insights into presenilin structure and function, and Alzheimer's disease. The applicants will identify mutations in the new presenilins, identify suppressors of these new presenilin mutants, perform structure/function studies, and look for genetic interactions with *lin-12*, *glp-1* and other genes.

References of Third Series of Experiments

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Greenwald, Iva
Levitan, Diane

(ii) TITLE OF INVENTION: IDENTIFICATION OF SEL-12 AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 9

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Cooper & Dunham LLP
(B) STREET: 1185 Avenue of the Americas
(C) CITY: New York
(D) STATE: New York
(E) COUNTRY: USA
(F) ZIP: 10036

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: White, John P.
(B) REGISTRATION NUMBER: 28,678
(C) REFERENCE/DOCKET NUMBER: 48231/JPW/AKC

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (212) 278-0400
(B) TELEFAX: (212) 391-0525

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 461 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..461

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Pro Ser Thr Arg Arg Gln Gln Glu Gly Gly Gly Ala Asp Ala Glu
1 5 10 15

Thr His Thr Val Tyr Gly Thr Asn Leu Ile Thr Asn Arg Asn Ser Gln
20 25 30

Glu Asp Glu Asn Val Val Glu Glu Ala Glu Leu Lys Tyr Gly Ala Ser

35 40 45

His Val Ile His Leu Phe Val Pro Val Ser Leu Cys Met Ala Leu Val
50 55 60

Val Phe Thr Met Asn Thr Ile Thr Phe Tyr Ser Gln Asn Asn Gly Arg
65 70 75 80

His Leu Leu Ser His Pro Phe Val Arg Glu Thr Asp Ser Ile Val Glu
85 90 95

Lys Gly Leu Met Ser Leu Gly Asn Ala Leu Val Met Leu Cys Val Val
100 105 110

Val Leu Met Thr Val Leu Leu Ile Val Phe Tyr Lys Tyr Lys Phe Tyr
115 120 125

Lys Leu Ile His Gly Trp Leu Ile Val Ser Ser Phe Leu Leu Leu Phe
130 135 140

Leu Phe Thr Thr Ile Tyr Val Gln Glu Val Leu Lys Ser Phe Asp Val
145 150 155 160

Ser Pro Ser Ala Leu Leu Val Leu Phe Gly Leu Gly Asn Tyr Gly Val
165 170 175

Leu Gly Met Met Cys Ile His Trp Lys Gly Pro Leu Arg Leu Gln Gln
180 185 190

Phe Tyr Leu Ile Thr Met Ser Ala Leu Met Ala Leu Val Phe Ile Lys
195 200 205

Tyr Leu Pro Glu Trp Thr Val Trp Phe Val Leu Phe Val Ile Ser Val
210 215 220

Trp Asp Leu Val Ala Val Leu Thr Pro Lys Gly Pro Leu Arg Tyr Leu
225 230 235 240

Val Glu Thr Ala Gln Glu Arg Asn Glu Pro Ile Phe Pro Ala Leu Ile
245 250 255

Tyr Ser Ser Gly Val Ile Tyr Pro Tyr Val Leu Val Thr Ala Val Glu
260 265 270

Asn Thr Thr Asp Pro Arg Glu Pro Thr Ser Ser Asp Ser Asn Thr Ser
275 280 285

Thr Ala Phe Pro Gly Glu Ala Ser Cys Ser Ser Glu Thr Pro Lys Arg
290 295 300

Pro Lys Val Lys Arg Ile Pro Gln Lys Val Gln Ile Glu Ser Asn Thr
305 310 315 320

Thr Ala Ser Thr Thr Gln Asn Ser Gly Val Arg Val Glu Arg Glu Leu
325 330 335

Ala Ala Glu Arg Pro Thr Val Gln Asp Ala Asn Phe His Arg His Glu
340 345 350

Glu Glu Glu Arg Gly Val Lys Leu Gly Leu Gly Asp Phe Ile Phe Tyr
355 360 365

Ser Val Leu Leu Gly Lys Ala Ser Ser Tyr Phe Asp Trp Asn Thr Thr
370 375 380

Ile Ala Cys Tyr Val Ala Ile Leu Ile Gly Leu Cys Phe Thr Leu Val
385 390 395 400

Leu Leu Ala Val Phe Lys Arg Ala Leu Pro Ala Leu Gln Phe Pro Phe
405 410 415

Ser Pro Asp Ser Phe Phe Thr Phe Val Pro Ala Gly Ser Ser Pro His
420 425 430

Leu Leu His Lys Ser Leu Lys Ser Val Tyr Tyr Ile Asn Ser Leu Phe
435 440 445

Leu Pro Phe Leu Cys Ile Ile Asn Phe Ser Ile Ile Ser
450 455 460

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 467 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: Active-site
(B) LOCATION: 1..467

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Thr Glu Leu Pro Ala Pro Leu Ser Tyr Phe Gln Asn Ala Gln Met
1 5 10 15

Ser Glu Asp Asn His Leu Ser Asn Thr Val Arg Ser Gln Asn Asp Asn
20 25 30

Arg Glu Arg Gln Glu His Asn Asp Arg Arg Ser Leu Gly His Pro Glu
35 40 45

Pro Leu Ser Asn Gly Arg Pro Gln Gly Asn Ser Arg Gln Val Val Glu
50 55 60

Gln Asp Glu Glu Glu Asp Glu Glu Leu Thr Leu Lys Tyr Gly Ala Lys
65 70 75 80

His Val Ile Met Leu Phe Val Pro Val Thr Leu Cys Met Val Val Val
85 90 95

Val Ala Thr Ile Lys Ser Val Ser Phe Tyr Thr Arg Lys Asp Gly Gln
100 105 110

Leu Ile Tyr Thr Pro Phe Thr Glu Asp Thr Glu Thr Val Gly Gln Arg
115 120 125

Ala Leu His Ser Ile Leu Asn Ala Ala Ile Met Ile Ser Val Ile Val
130 135 140

Val Met Thr Ile Leu Leu Val Val Leu Tyr Lys Tyr Arg Cys Tyr Lys
145 150 155 160

Val Ile His Ala Trp Leu Ile Ile Ser Ser Leu Leu Leu Leu Phe Phe
165 170 175

Phe Ser Phe Ile Tyr Leu Gly Glu Val Phe Lys Thr Tyr Asn Val Ala
180 185 190

Val Asp Tyr Val Thr Val Ala Leu Leu Ile Trp Asn Phe Gly Val Val
195 200 205

Gly Met Ile Ser Ile His Trp Lys Gly Pro Leu Arg Leu Gln Gln Ala
210 215 220

Tyr Leu Ile Met Ile Ser Ala Leu Met Ala Leu Val Phe Ile Lys Tyr
225 230 235 240

Leu Pro Glu Trp Thr Ala Trp Leu Ile Leu Ala Val Ile Ser Val Tyr
245 250 255

Asp Leu Val Ala Val Leu Cys Pro Lys Gly Pro Leu Arg Met Leu Val
260 265 270

Glu Thr Ala Gln Glu Arg Asn Glu Thr Leu Phe Pro Ala Leu Ile Tyr
275 280 285

Ser Ser Thr Met Val Trp Leu Val Asn Met Ala Glu Gly Asp Pro Glu
290 295 300

Ala Gln Arg Arg Val Ser Lys Asn Ser Lys Tyr Asn Ala Glu Ser Thr
305 310 315 320

Glu Arg Glu Ser Gln Asp Thr Val Ala Glu Asn Asp Asp Gly Gly Phe
325 330 335

Ser Glu Glu Trp Glu Ala Gln Arg Asp Ser His Leu Gly Pro His Arg
340 345 350

Ser Thr Pro Glu Ser Arg Ala Ala Val Gln Glu Leu Ser Ser Ser Ile
355 360 365

Leu Ala Gly Glu Asp Pro Glu Glu Arg Gly Val Lys Leu Gly Leu Gly
370 375 380

Asp Phe Ile Phe Tyr Ser Val Leu Val Gly Lys Ala Ser Ala Thr Ala
385 390 395 400

Ser Gly Asp Trp Asn Thr Thr Ile Ala Cys Phe Val Ala Ile Leu Ile
405 410 415

Gly Leu Cys Leu Thr Leu Leu Leu Leu Ala Ile Phe Lys Lys Ala Leu
420 425 430

Pro Ala Leu Pro Ile Ser Ile Thr Phe Gly Leu Val Phe Tyr Phe Ala
435 440 445

Thr Asp Tyr Leu Val Gln Pro Phe Met Asp Gln Leu Ala Phe His Gln
450 455 460

Phe Tyr Ile
465

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 157 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: Active-site
(B) LOCATION: 1..157

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Glu Gly Lys Ser Pro Ser Asn Thr Glu Arg Xaa Val Ile Met Leu Phe
1 5 10 15

Val Pro Val Thr Leu Cys Met Ile Val Val Val Ala Thr Ile Lys Ser
20 25 30

Val Arg Phe Tyr Thr Glu Lys Asn Gly Gln Leu Ile Tyr Thr Pro Phe
35 40 45

Thr Glu Asp Thr Pro Ser Val Gly Gln Arg Leu Leu Asn Ser Val Leu
50 55 60

Asn Thr Leu Ile Met Ile Ser Val Ile Val Val Met Thr Ile Phe Leu
65 70 75 80

Val Val Leu Tyr Lys Tyr Arg Cys Tyr Lys Phe Ile His Gly Trp Leu
85 90 95

Ile Met Ser Ser Leu Met Leu Leu Phe Leu Phe Thr Tyr Ile Tyr Leu
100 105 110

Gly Glu Val Leu Lys Thr Tyr Asn Val Ala Met Asp Tyr Pro Thr Leu
115 120 125

Leu Leu Thr Val Trp Glu Leu Arg Gly Ser Gly His Gly Val His Pro
130 135 140

Leu Glu Gly Ala Phe Gly Ala Ala Glu Ala Tyr Leu Ser
145 150 155

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 465 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: Active-site
(B) LOCATION: 1..465

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Asp Thr Leu Arg Ser Ile Ser Ser Glu Leu Val Arg Ser Ser Gln
1 5 10 15

Leu Arg Trp Thr Leu Phe Ser Val Ile Ala Asn Met Ser Leu Thr Leu
20 25 30

Ser Ile Trp Ile Gly Val Tyr Asn Met Glu Val Asn Ser Glu Leu Ser
35 40 45

Lys Thr Tyr Phe Leu Asp Pro Ser Phe Glu Gln Thr Thr Gly Asn Leu
50 55 60

Leu Leu Asp Gly Phe Ile Asn Gly Val Gly Thr Ile Leu Val Leu Gly
65 70 75 80

Cys Val Ser Phe Ile Met Leu Ala Phe Val Leu Phe Asp Phe Arg Arg

85 90 95

Ile Val Lys Ala Trp Leu Thr Leu Ser Cys Leu Leu Ile Leu Phe Gly
100 105 110

Val Ser Ala Gln Thr Leu His Asp Met Phe Ser Gln Val Phe Asp Gln
115 120 125

Asp Asp Asn Asn Gln Tyr Tyr Met Thr Ile Val Leu Ile Val Val Pro
130 135 140

Thr Val Val Tyr Gly Phe Gly Gly Ile Tyr Ala Phe Phe Ser Asn Ser
145 150 155 160

Ser Leu Ile Leu His Gln Ile Phe Val Val Thr Asn Cys Ser Leu Ile
165 170 175

Ser Val Phe Tyr Leu Arg Val Phe Pro Ser Lys Thr Thr Trp Phe Val
180 185 190

Leu Trp Ile Val Leu Phe Trp Asp Leu Phe Ala Val Leu Ala Pro Met
195 200 205

Gly Pro Leu Lys Lys Val Gln Glu Lys Ala Ser Asp Tyr Ser Lys Cys
210 215 220

Val Leu Asn Leu Ile Met Phe Ser Ala Asn Glu Lys Arg Leu Thr Ala
225 230 235 240

Gly Ser Asn Gln Glu Glu Thr Asn Glu Gly Glu Glu Ser Thr Ile Arg
245 250 255

Arg Thr Val Lys Gln Thr Ile Glu Tyr Tyr Thr Lys Arg Glu Ala Gln
260 265 270

Asp Asp Glu Phe Tyr Gln Lys Ile Arg Gln Arg Arg Ala Ala Ile Asn
275 280 285

Pro Asp Ser Val Pro Thr Glu His Ser Pro Leu Val Glu Ala Glu Pro
290 295 300

Ser Pro Ile Glu Leu Lys Glu Lys Asn Ser Thr Glu Glu Leu Ser Asp
305 310 315 320

Asp Glu Ser Asp Thr Ser Glu Thr Ser Ser Gly Ser Ser Asn Leu Ser
325 330 335

Ser Ser Asp Ser Ser Thr Thr Val Ser Thr Ser Asp Ile Ser Thr Ala
340 345 350

Glu Glu Cys Asp Gln Lys Glu Trp Asp Asp Leu Val Ser Asn Ser Leu
355 360 365

Pro Asn Asn Asp Lys Arg Pro Ala Thr Ala Ala Asp Ala Leu Asn Asp
370 375 380

Gly Glu Val Leu Arg Leu Gly Phe Gly Asp Phe Val Phe Tyr Ser Leu
385 390 395 400

Leu Ile Gly Gln Ala Ala Ala Ser Gly Cys Pro Phe Ala Val Ile Ser
405 410 415

Ala Ala Leu Gly Ile Leu Phe Gly Leu Val Val Thr Leu Thr Val Phe
420 425 430

Ser Thr Glu Glu Ser Thr Thr Pro Ala Leu Pro Leu Pro Val Ile Cys
435 440 445

Gly Thr Phe Cys Tyr Phe Ser Ser Met Phe Phe Trp Glu Gln Leu Tyr
450 455 460

Gly
465

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1500 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GTTTAATTAC CCAAGTTTGA GATGCCTTCC ACAAGGAGAC AACAGGAGGG CGGAGGTGCA 60

GATGCGGAAA CACATACCGT TTACGGTACA AATCTGATAA CAAATCGGAA TAGCCAAGAA 120

GACGAAAATG TTGTGGAAGA AGCGGAGCTG AAATACGGAG CATCTCACGT TATTCATCTA 180

TTTGTGCCGG TGTCACTATG CATGGCTCTG GTTGTTTTTA CGATGAACAC GATTACGTTT 240

TATAGTCAAA ACAATGGAAG GCATTTACTA TCACATCCTT TTGTCCGGGA AACAGACAGT 300

ATCGTTGAGA AGGGATTGAT GTCACTTGGA AATGCTCTCG TCATGTTGTG CGTGGTCGTT 360

CTGATGACAG TTCTGCTGAT TGTTTTCTAT AAATACAAGT TTTATAAGCT TATTCATGGA 420

TGGCTTATTG TCAGCAGTTT TCTTCTTCTT TTCCTATTCA CTACAATCTA TGTGCAAGAA 480

GTTCTGAAAA GTTTCGATGT GTCTCCCAGC GCACTATTGG TTTTGTTTGG ACTGGGTAAC 540

TATGGAGTTC TCGGAATGAT GTGTATACAT TGGAAAGGTC CATTGCGTCT GCAACAGTTC 600

TACCTTATTA CAATGTCTGC ACTAATGGCT CTGGTCTTTA TCAAGTACCT ACCAGAATGG 660

ACTGTGTGGT TTGTGCTGTT TGTTATCTCG GTTTGGGATC TGGTTGCCGT GCTCACACCA 720

AAAGGACCAT TGAGATATTT GGTGGAAACT GCACAGGAGA GAAACGAGCC AATTTTCCCG 780

GCGCTGATTT ATTCGTCTGG AGTCATCTAT CCCTACGTTC TTGTTACTGC AGTTGAAAAC 840

ACGACAGACC CCCGTGAACC GACGTCGTCA GACTCAAATA CTTCTACAGC TTTTCCTGGA 900

GAGGCGAGTT GTTCATCTGA AACGCCAAAA CGGCCAAAAG TGAAACGAAT TCCTCAAAAA 960

GTGCAAATCG AATCGAATAC TACAGCTTCA ACGACACAAA ACTCTGGAGT AAGGGTGGAA 1020

CGGGAGCTAG CTGCTGAGAG ACCAACTGTA CAAGACGCCA ATTTTCACAG GCACGAAGAG 1080

GAAGAGAGAG GTGTGAAACT TGGTCTGGGC GACTTCATTT TCTACTCTGT TCTCCTCGGC 1140

AAGGCTTCAT CGTACTTTGA CTGGAACACG ACTATCGCTT GTTATGTGGC CATTCTTATC 1200

GGTCTCTGCT TCACTCTTGT CCTGCTCGCC GTCTTCAAAC GAGCACTCCC GGCTCTGCAA 1260

TTTCCATTTT CTCCGGACTC ATTTTTTACT TTTGTACCCG CTGGATCATC ACCCCATTTG 1320

TTACACAAGT CTCTCAAAAG TGTTTATTAT ATTAATTCTC TGTTTTTGCC ATTTCTTTGC 1380

ATCATCAACT TTTCGATTAT ATCTTGAGCG ATCTCAAAGC TTTATTTTAC ATACCTATTT 1440

ATTTTTGAAC TTTGTCATTT AAGTTATATA AATAATTTAT TAAAAAAAAA AAAAAAAAAA 1500

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 461 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: Active-site
(B) LOCATION: 1..461

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Pro Ser Thr Arg Arg Gln Gln Glu Gly Gly Gly Ala Asp Ala Glu
1 5 10 15

Thr His Thr Val Tyr Gly Thr Asn Leu Ile Thr Asn Arg Asn Ser Gln
20 25 30

Glu Asp Glu Asn Val Val Glu Glu Ala Glu Leu Lys Tyr Gly Ala Ser
35 40 45

His Val Ile His Leu Phe Val Pro Val Ser Leu Cys Met Ala Leu Val
50 55 60

Val Phe Thr Met Asn Thr Ile Thr Phe Tyr Ser Gln Asn Asn Gly Arg
65 70 75 80

His Leu Leu Ser His Pro Phe Val Arg Glu Thr Asp Ser Ile Val Glu
85 90 95

Lys Gly Leu Met Ser Leu Gly Asn Ala Leu Val Met Leu Cys Val Val
100 105 110

Val Leu Met Thr Val Leu Leu Ile Val Phe Tyr Lys Tyr Lys Phe Tyr
115 120 125

Lys Leu Ile His Gly Trp Leu Ile Val Ser Ser Phe Leu Leu Leu Phe
130 135 140

Leu Phe Thr Thr Ile Tyr Val Gln Glu Val Leu Lys Ser Phe Asp Val
145 150 155 160

Ser Pro Ser Ala Leu Leu Val Leu Phe Gly Leu Gly Asn Tyr Gly Val
165 170 175

Leu Gly Met Met Cys Ile His Trp Lys Gly Pro Leu Arg Leu Gln Gln
180 185 190

Phe Tyr Leu Ile Thr Met Ser Ala Leu Met Ala Leu Val Phe Ile Lys
195 200 205

Tyr Leu Pro Glu Trp Thr Val Trp Phe Val Leu Phe Val Ile Ser Val
210 215 220

Trp Asp Leu Val Ala Val Leu Thr Pro Lys Gly Pro Leu Arg Tyr Leu
225 230 235 240

Val Glu Thr Ala Gln Glu Arg Asn Glu Pro Ile Phe Pro Ala Leu Ile
245 250 255

Tyr Ser Ser Gly Val Ile Tyr Pro Tyr Val Leu Val Thr Ala Val Glu
260 265 270

Asn Thr Thr Asp Pro Arg Glu Pro Thr Ser Ser Asp Ser Asn Thr Ser
275 280 285

Thr Ala Phe Pro Gly Glu Ala Ser Cys Ser Ser Glu Thr Pro Lys Arg
290 295 300

Pro Lys Val Lys Arg Ile Pro Gln Lys Val Gln Ile Glu Ser Asn Thr
305 310 315 320

Thr Ala Ser Thr Thr Gln Asn Ser Gly Val Arg Val Glu Arg Glu Leu
325 330 335

Ala Ala Glu Arg Pro Thr Val Gln Asp Ala Asn Phe His Arg His Glu
340 345 350

Glu Glu Glu Arg Gly Val Lys Leu Gly Leu Gly Asp Phe Ile Phe Tyr
355 360 365

Ser Val Leu Leu Gly Lys Ala Ser Ser Tyr Phe Asp Trp Asn Thr Thr
370 375 380

Ile Ala Cys Tyr Val Ala Ile Leu Ile Gly Leu Cys Phe Thr Leu Val
385 390 395 400

Leu Leu Ala Val Phe Lys Arg Ala Leu Pro Ala Leu Gln Phe Pro Phe
405 410 415

Ser Pro Asp Ser Phe Phe Thr Phe Val Pro Ala Gly Ser Ser Pro His
420 425 430

Leu Leu His Lys Ser Leu Lys Ser Val Tyr Tyr Ile Asn Ser Leu Phe
435 440 445

Leu Pro Phe Leu Cys Ile Ile Asn Phe Ser Ile Ile Ser
450 455 460

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TGTCTGAGTT ACTAGTTTTC C 21

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGAATCTGAA GCACCTGTAA GCAT 24

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 448 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Leu Thr Phe Met Ala Ser Asp Ser Glu Glu Glu Val Cys Asp Glu
1 5 10 15

Arg Thr Ser Leu Met Ser Ala Glu Ser Pro Thr Pro Arg Ser Cys Gln
20 25 30

Glu Gly Arg Gln Gly Pro Glu Asp Gly Glu Asn Thr Ala Gln Trp Arg
35 40 45

Ser Gln Glu Asn Glu Glu Asp Gly Glu Glu Asp Pro Asp Arg Tyr Val
50 55 60

Cys Ser Gly Val Pro Gly Arg Pro Pro Gly Leu Glu Glu Glu Leu Thr
65 70 75 80

Leu Lys Tyr Gly Ala Lys His Val Ile Met Leu Phe Val Pro Val Thr
85 90 95

Leu Cys Met Ile Val Val Val Ala Thr Ile Lys Ser Val Arg Phe Tyr
100 105 110

Thr Glu Lys Asn Gly Gln Leu Ile Tyr Thr Pro Phe Thr Glu Asp Thr
115 120 125

Pro Ser Val Gly Gln Arg Leu Leu Asn Ser Val Leu Asn Thr Leu Ile
130 135 140

Met Ile Ser Val Ile Val Val Met Thr Ile Phe Leu Val Val Leu Tyr
145 150 155 160

Lys Tyr Arg Cys Tyr Lys Phe Ile His Gly Trp Leu Ile Met Ser Ser
165 170 175

Leu Met Leu Leu Phe Leu Phe Thr Tyr Ile Tyr Leu Gly Glu Val Leu
180 185 190

Lys Thr Tyr Asn Val Ala Met Asp Tyr Pro Thr Leu Leu Leu Thr Val
195 200 205

Trp Asn Phe Gly Ala Val Gly Met Val Cys Ile His Trp Lys Gly Pro
210 215 220

Leu Val Leu Gln Gln Ala Tyr Leu Ile Met Ile Ser Ala Leu Met Ala
225 230 235 240

Leu Val Phe Ile Lys Tyr Leu Pro Glu Trp Ser Ala Trp Val Ile Leu
245 250 255

Gly Ala Ile Ser Val Tyr Asp Leu Val Ala Val Leu Cys Pro Lys Gly
260 265 270

Pro Leu Arg Met Leu Val Glu Thr Ala Gln Glu Arg Asn Glu Pro Ile
275 280 285

Phe Pro Ala Leu Ile Tyr Ser Ser Ala Met Val Trp Thr Val Gly Met
290 295 300

Ala Lys Leu Asp Pro Ser Ser Gln Gly Ala Leu Gln Leu Pro Tyr Asp
305 310 315 320

Pro Glu Met Glu Glu Asp Ser Tyr Asp Ser Phe Gly Glu Pro Ser Tyr
325 330 335

Pro Glu Val Phe Glu Pro Pro Leu Thr Gly Tyr Pro Gly Glu Glu Leu
340 345 350

Glu Glu Glu Glu Glu Arg Gly Val Lys Leu Gly Leu Gly Asp Phe Ile
355 360 365

Phe Tyr Ser Val Leu Val Gly Lys Ala Ala Ala Thr Gly Ser Gly Asp
370 375 380

Trp Asn Thr Thr Leu Ala Cys Phe Val Ala Ile Leu Ile Gly Leu Cys
385 390 395 400

Leu Thr Leu Leu Leu Leu Ala Val Phe Lys Lys Ala Leu Pro Ala Leu
405 410 415

Pro Ile Ser Thr Thr Phe Gly Leu Ile Phe Tyr Phe Ser Thr Asp Asn
420 425 430

Leu Val Arg Pro Phe Met Asp Thr Leu Ala Ser His Gln Leu Tyr Ile
435 440 445

What is claimed is:

1. An isolated nucleic acid molecule encoding a SEL-12.

2. An isolated nucleic acid molecule encoding a mutated SEL-12.

3. An isolated nucleic acid molecule of claim 2, wherein the mutated SEL-12 contains at least one of the following: leucine at position 115, arginine at position 132, glutamic acid at position 215, valine at position 229, valine at position 254, valine at position 255, valine at position 371, tyrosine at position 387, isoleucine at position 104 or valine at position 204.

4. An isolated nucleic acid molecule of claim 2, wherein the mutated SEL-12 contains one or more alterations.

5. An isolated nucleic acid molecule encoding a *Caenorhabditis elegans* protein that is homologous to SEL-12.

6. An isolated DNA molecule of claim 2 or 3, wherein the mutation is generated by in vitro mutagenesis.

7. An isolated DNA molecule of any of claim 1 to 6.

8. An isolated cDNA molecule of claim 7.

9. An isolated genomic DNA molecule of claim 7.

10. An isolated RNA molecule of any of claim 1 to 6.

11. An isolated nucleic acid molecule of claim 1, wherein the SEL-12 has substantially the same amino acid sequence as the amino acid sequence shown in Figure 1A.

12. A nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a unique sequence within

the sequence of a nucleic acid molecule of claim 1.

13. A DNA molecule of claim 12.

14. An RNA molecule of claim 12.

15. A vector which comprises the isolated nucleic acid molecule of claim 1.

16. An isolated nucleic acid molecule of claim 7, 8 or 9 operatively linked to a promoter of RNA transcription.

17. The vector of claim 15 or 16, wherein the vector is a plasmid.

18. The plasmid of claim 17 designated pMX8 (ATCC Accession No. 97278).

19. The plasmid of claim 17 designated p1-1E (ATCC Accession No. 97279).

20. A host vector system for the production of a SEL-12 protein which comprises the vector of claim 15 and a suitable host.

21. A host vector system of claim 20, wherein the suitable host is a bacterial cell, insect cell, plant or mammalian cell.

22. A purified SEL-12 protein or a fragment thereof.

23. A purified mutated SEL-12 protein or a fragment thereof.

24. A method for production of an antibody comprising:

a) administering an amount of the purified protein or fragment of SEL-12 or mutated SEL-12 to a suitable animal effective to produce an antibody against SEL-12 or mutated SEL-12 protein in the animal; and

b) recovering the produced antibody so produced from the

animal.

25. A method for production of an antibody capable of binding to wild-type or mutant S182 or E5-1/STM2, wherein the antibody is produced by in vitro immunization.

26. A method for production of an antibody capable of binding to wild-type or mutant S182 or E5-1/STM2, wherein the antibody is produced by screening a differential phage display library.

27. A method for production of an antibody capable of binding to wild-type or mutant S182 or E5-1/STM2 comprising:

a) determining conserved regions revealed by alignment of the SEL-12, S182 and E5-1/STM2 protein sequences;

b) synthesizing peptides corresponding to the revealed conserved regions;

c) administering an amount of the synthesized peptides to a suitable animal effective to produce an antibody against the peptides in the animal; and

b) recovering the produced antibody so produced from the animal.

28. An antibody produced by the method of any of claim 24 to 27.

29. A monoclonal antibody of claim 28.

30. A transgenic animal comprising a DNA molecule of any of claims 7 to 9.

31. The transgenic animal of claim 30 wherein the animal is a *Caenorhabditis elegans*.

32. A transgenic *Caenorhabditis elegans* animal comprising wild-type or mutant human S182 gene.

33. A transgenic *Caenorhabditis elegans* animal comprising wild-type or mutant human STM2/E5-1 gene.

34. A transgenic *Caenorhabditis elegans* animal comprising wild-type or mutant human presenilin gene.

35. A transgenic *Caenorhabditis elegans* animal of any of claim 30-34, wherein the wild-type or mutant human S182, or wild-type or mutant STM2/E5-1 gene, or mutant human presenilin gene is under the control of *sel-12* or *lin-12* regulatory sequence.

36. A transgenic *Caenorhabditis elegans* animal of claim 30-34, wherein the wild-type or mutant human S182, or wild-type or mutant STM2/E5-1 gene, or mutant human presenilin gene is under the control of a regulatory sequence other than the *sel-12* or *lin-12* regulatory sequence.

37. A transgenic *Caenorhabditis elegans* animal of claim 30-36 having an egg-laying constitutive (Egl^c) phenotype.

38. A transgenic *Caenorhabditis elegans* animal of claim 30-36 having a phenotype other than egg-laying constitutive (Egl^c).

39. A transgenic *Caenorhabditis elegans* animal having a *sel-12* allele that reduces, eliminates or elevates *sel-12* activity.

40. A transgenic *Caenorhabditis elegans* animal having a *sel-12* transgene carrying a mutation that is equivalent to a mutation that causes Alzheimer's disease [*sel-12(Alz)*].

41. A method for identifying a compound which is capable of ameliorating Alzheimer disease comprising administering effective amount of the compound to the transgenic animal of any of claim 30-40, the alteration of the conditions of the transgenic animal indicating the compound is capable of ameliorating Alzheimer's disease.

42. A method of claim 41, wherein at least one signalling pathway is altered.

43. A method of claim 42, wherein the signalling pathway is a neuronal signalling pathway.

44. A method of claim 43, wherein the signalling pathway is the serotonergic signalling pathway.

45. A previously unknown compound identified by the method of any of claim 41-44.

46. A pharmaceutical composition comprising an effective amount of the compound identified by the method of claim of any of 41-44 and a pharmaceutically acceptable carrier.

47. A method for determining whether a compound is capable of ameliorating Alzheimer's disease comprising:

a) treating *Caenorhabditis elegans* mutants having reduced, increased or altered *sel-12* activity with the compound; and

b) determining whether the compound suppresses, enhances or has no effect on the phenotype of the mutant, the suppression or enhancement of the phenotype indicating that the compound is capable of ameliorating Alzheimer's disease.

48. A method of claim 47, wherein the *Caenorhabditis elegans* mutant to be treated is *sel-12(ar171)* (ATCC Accession No. 97292).

49. A method of claim 47, wherein the *Caenorhabditis elegans* mutant to be treated is *sel-12(ar131)* (ATCC Accession No. 97293).

50. A method of claim 47, wherein the *Caenorhabditis elegans* mutant to be treated is a *sel-12* allele that reduces or eliminates *sel-12* activity.

51. A method of claim 47, wherein the *Caenorhabditis elegans* mutant to be treated is a *sel-12* allele that elevates or alters *sel-12* activity.

52. A method of claim 47, wherein the *Caenorhabditis elegans* mutant to be treated is a *sel-12* transgenic animal carrying a mutation in *sel-12* that is equivalent to a mutation that causes Alzheimer's disease [*sel-12(Alz)*].

53. A method of claim 47, wherein the *Caenorhabditis elegans* mutant to be treated is a *sel-12* transgenic animal carrying a mutation in *sel-12*, and results in an Egl^c phenotype.

54. A method of claim 47, wherein the *Caenorhabditis elegans* mutant to be treated is a *sel-12* transgenic animal carrying a mutation in *sel-12* that is equivalent to a mutation that causes Alzheimer's disease, and results in a phenotype other than Egl^c phenotype.

55. A method of claim 47, wherein the *Caenorhabditis elegans* mutant to be treated is a transgenic animal from any of claim 30-40.

56. A previously unknown compound determined by the method of any of claim 47-55 to be capable of ameliorating Alzheimer's disease.

57. A pharmaceutical composition comprising an effective amount of the compound determined by the method of claim 47-55 to be capable of ameliorating Alzheimer's disease and a pharmaceutically acceptable carrier.

58. A method for identifying a suppressor of the multivulva phenotype of *lin-12* gain-of-function mutation comprising:

a) mutagenizing *lin-12 Caenorhabditis elegans* worms with an effective amount of an appropriate mutagen;

b) screening for revertants in the F1, F2 and F3 generations; and

c) isolating the screened revertant, thereby identifying a suppressor of the multivulva phenotype of *lin-12* gain-of-function mutation.

59. A suppressor identified by method of claim 58.

60. An animal having a suppressor of claim 59, designated *sel-12(ar131)* (ATCC Accession No. 97293).

61. An animal having a suppressor of claim 59, designated *sel-12(ar133)*.

62. A method for identifying a mutant *sel-12* gene which reduces *sel-12* function comprising:

a) mutagenizing *Caenorhabditis elegans* worms with an effective amount of an appropriate mutagen;

b) performing complementation screening of the mutagenized worms to determine if a descendant of a mutagenized worm bears a mutation that fails to complement a suppressor of claim 59 for the Egl defect; and

c) isolating the individual worm and determining the phenotype of worms carrying the new allele in its homozygous form and *in trans* to a deficiency, thereby identifying a mutant *sel-12* gene which reduces *sel-12* function.

63. A method for identifying a mutant *sel-12* gene which reduces or elevates *sel-12* function comprising:

a) mutagenizing *Caenorhabditis elegans* worms with an effective amount of an appropriate mutagen;

b) identifying suppressors or enhancers of *daf-1* single mutants, or *daf-1; sel-12* double mutants, or mutations in other genes that interact with *sel-12;*

c) isolating the individual worm and determining the phenotype of worms carrying the new allele in its homozygous form and *in trans* to a deficiency, thereby identifying a mutant *sel-12* gene which reduces *sel-12* function.

64. A method of claim 63, further comprising performing DNA sequence analysis of the identified mutant *sel-12* gene to determine the molecular lesion responsible for the

mutation.

65. A mutant *sel-12* gene identified by the method of any of claim 62-64.

66. An animal having a mutant *sel-12* gene of claim 62, designated *sel-12 (ar171)* (ATCC Accession No. 97292).

67. A method for producing extragenic suppressors or enhancers of a *sel-12* allele comprising:
a) mutagenizing *sel-12* mutant hermaphrodites with an effective amount of a mutagen;
b) screening for revertants in the F1, F2 and F3 generations; and
c) isolating the screened revertant, thereby producing extragenic suppressors or enhancers of a *sel-12* allele.

68. A method for producing extragenic suppressors of a *sel-12* allele comprising:
a) mutagenizing *sel-12(ar171) or sel-12(ar131)* mutant hermaphrodites with an effective amount of a mutagen;
b) screening for revertants in the F1, F2 and F3 generations; and
c) isolating the screened revertant, thereby producing extragenic suppressors or enhancers of a *sel-12* allele.

69. A method for producing extragenic suppressors or enhancers of a *sel-12* allele comprising:
a) mutagenizing *daf-1(m213); sel-12(ar171)* mutant hermaphrodites with an effective amount of a mutagen;
b) screening for revertants in the F1, F2 and F3 generations; and
c) isolating the screened revertant, thereby producing extragenic suppressors or enhancers of a *sel-12* allele.

70. A method for producing extragenic suppressors or enhancers

of a *sel-12(Alz)* mutant comprising:

a) mutagenizing *sel-12 (Alz)* hermaphrodites with an effective amount of a mutagen;

b) screening for revertants in the F1, F2 and F3 generations; and

c) isolating the screened revertant, thereby producing extragenic suppressors or enhancers of a *sel-12(Alz)* mutant.

71. A suppressor or enhancers produced by the method of any of claim 67-70.

72. A suppressor of presenilin, designated *spr-1, spr-2, spr-3 or spr-4*.

73. The human homolog of *spr-1, spr-2, spr-3 or spr-4*.

74. A human homolog of a gene defined by extragenic suppressor or enhancer of a *sel-12* mutant.

75. A Drosophila homolog of a gene defined by extragenic suppressors of a *sel-12* mutant.

76. A mouse homolog of a gene defined by extragenic suppressor of a *sel-12* mutant.

77. The homolog of any of claim 73-76, wherein the *sel-12* mutant is *sel-12(ar171)* (ATCC Accession No. 97292).

78. The homolog of any of claim 73-76, wherein the *sel-12* mutant is *sel-12(Alz)* transgene.

79. The homolog of any of claim 73-76, wherein the *sel-12* mutant is *sel-12(ar131)* (ATCC Accession No. 97293)

80. The homolog of any of claim 73-76, wherein the *sel-12* mutant is any other *sel-12* allele.

81. A method for identifying a suppressor gene comprising

performing DNA sequence analysis of the suppressor of claim 68 to identify the suppressor gene.

82. The suppressor gene identified by method of claim 81.

83. A human suppressor gene of claim 82.

84. A Drosophila suppressor gene of claim 82.

85. A mouse suppressor gene of claim 82.

86. The method of any of claim 59, 60, 61, 65, 66 or 67, wherein the mutagen is ethyl methanesulfonate.

FIGURE 1A

```
1    GTTTAATTACCCAAGTTTGAGATGCCTTCCACAAGGAGACAACAGGAGGGCGGAGGTGCAGATGCGGAAACACAtACCGTTTACGGTACAAATCTGATAA   100
1                    M  P  S  T  R  R  Q  Q  E  G  G  G  A  D  A  E  T  H  T  V  Y  G  T  N  L  I  T     27
1    CAAATCGGAATAGCCAAGAAGACGAAAATGTTGTGGAAGAAGCGGAGCTGAAATACGGAGCATCTCACGTTATTCATCTATTTGTGCCGGTGTCACTATG   200
101  N  R  N  S  Q  E  D  E  N  V  V  E  E  A  E  L  K  Y  G  A  S  H  V  I  H  L  F  V  P  V  S  L  C      60
28   CATGGCTCTGGTTGTTTTTACGATGAACACGATTACGTTTTATAGTCAAAACAATGGAAGGCATTcACTATCACATCCTTTTGTCCGGGAAACAGACAGT   300
201  M  A  L  V  V  F  T  M  N  T  I  T  F  Y  S  Q  N  N  G  R  H  L  L  S  H  P  F  V  R  E  T  D  S      93
61   ATCGTTGAGAAGGGATTGATGTCACTTGGAAATGCTCTCGTCATGTTGTGCGTGGTCGTTCTGATGACAGTTCTGCTGATTGTTTTCTATAAATACAAGT   400
301  I  V  E  K  G  L  M  S  L  G  N  A  L  V  M  L  C  V  V  V  L  M  T  V  L  L  I  V  F  Y  K  Y  K     126
94   TTTATAAGCTTATTCATGGATGGCTTATTGTCAGCAGTTTTCTTCTTCTTTTCCTATTCACTACAATCTATGTGCaAGAAGTTCTGAAAAGTTTCGATGT   500
401  F  Y  K  L  I  H  G  W  L  I  V  S  S  F  L  L  L  F  L  F  T  T  I  Y  V  Q  E  V  L  K  S  F  D  V     160
127  GTCTCCCAGCGCACTATTGGTTTTGTTTGGACTGGGTAACTATGGAGTTCTCGGAATGATGTGTATACATTGGAAAGGTCCATTGCGTCTGCAACAGTTC   600
501  S  P  S  A  L  L  V  L  F  G  L  G  N  Y  G  V  L  G  M  M  C  I  H  W  K  G  P  L  R  L  Q  Q  F     193
161  TACCTTATTACAATGTCTGCACTAATGGCTCTGGTCTTTATCAAGTACCTACCAGAATGGACTGTGTGGTTTGTGCTGTTTGTTATCTCGGTTTGGGATC   700
601  Y  L  I  T  M  S  A  L  M  A  L  V  F  I  K  Y  L  P  E  W  T  V  W  F  V  L  F  V  I  S  V  W  D  L     227
194  TGGTTGCCGTGCTCACACCAAAAGGACCATTGAGATATTTGGTGGAAACTGCACAGGAGAGAAACGAGCCAATTTTCCCGGCGCTGATTTATTCGtCTGG   800
701  V  A  V  L  T  P  K  G  P  L  R  Y  L  V  E  T  A  Q  E  R  N  E  P  I  F  P  A  L  I  Y  S  S  G     260
228  AGTCATCTATCCCTACGTTCTTGTTACTGCAGTTGAAAACACGACAGACCCCCGTGAACCGACGTCGTCAGACTCAAATaCTTCTACAGCTTTTCCTGGA   900
801  V  I  Y  P  Y  V  L  V  T  A  V  E  N  T  T  D  P  R  E  P  T  S  S  D  S  N  T  S  T  A  F  P  G     293
261  GAGGCGAGTTGTTCATCTGAAACGCCAAAACGGCCAAAAGTGAAACGAATTCCTCAAAAAGTGCAAATCGAATCGAATACTACAGCTTCAACGACACAAA  1000
901  E  A  S  C  S  S  E  T  P  K  R  P  K  V  K  R  I  P  Q  K  V  Q  I  E  S  N  T  T  A  S  T  T  Q  N     327
294  ACTCTGGAGTAAGGGTGGAACGGGAGCTAGCTGCTGAGAGACCAACTGTACAAGACGCCAATTTTCACAGGCACGAAGAGGAAGAGaGAGGTGTGAAACT  1100
1001 S  G  V  R  V  E  R  E  L  A  A  E  R  P  T  V  Q  D  A  N  F  H  R  H  E  E  E  E  R  G  V  K  L     360
328  TGGTCTGGGCGACTTCATTTTCTACTCTGTTCTCCTCGGCAAGGCTTCATCGTACTTTGACTGGAACACGACTATCGCTTGTTATGTGGCCATTCTTATC  1200
1101 G  L  G  D  F  I  F  Y  S  V  L  L  G  K  A  S  S  Y  F  D  W  N  T  T  I  A  C  Y  V  A  I  L  I     393
361  GGTCTCTGCTTCACTCTTGTCCTGCTCGCCGTCTTCAAACGAGCACTCCCGGCTCTGCAATTTCCATTTTCTCCGGACTCATTTTTTACTTTTGTACCCG  1300
1201 G  L  C  F  T  L  V  L  L  A  V  F  K  R  A  L  P  A  L  Q  F  P  F  S  P  D  S  F  F  T  F  V  P  A  427
394  CTGGATCATCACCCCATTTGTTACACAAGTCTCTCAAAAGTGTTTATTATATTAATTCTCTGTTTTTGCCATTTCTTTGCATCATCAACTTTTCGATTAT  1400
1301 G  L  C  F  T  L  V  L  L  A  V  F  K  R  A  L  P  A  L  Q  F  P  F  S  P  D  S  F  F  T  F  V  P  A  460
428  ATCTTGAGCGATCTCAAAGCTTTATTTTACATACCTATTTATTTTTGAACTTTGTCATTTAAGTTATATAAATAATTTATTAAAAAAAAAAAAAAAAAAA  1500
1401 G  S  S  P  H  L  L  H  K  S  L  K  S  V  Y  Y  I  N  S  L  F  L  P  F  L  C  I  I  N  F  S  I  I     461
461  S  *
```

FIGURE 1B

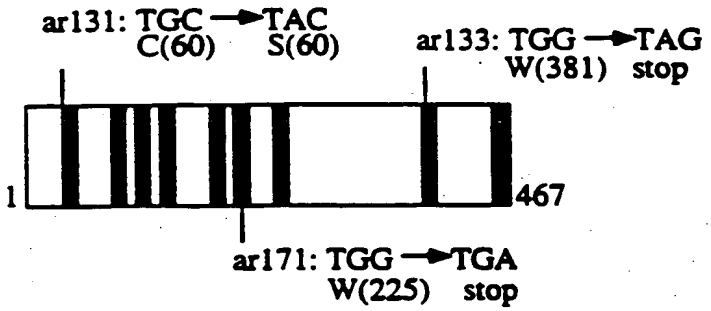
ar131: TGC → TAC
C(60) S(60)
ar133: TGG → TAG
W(381) stop
1
467
ar171: TGG → TGA
W(225) stop

FIGURE 2A

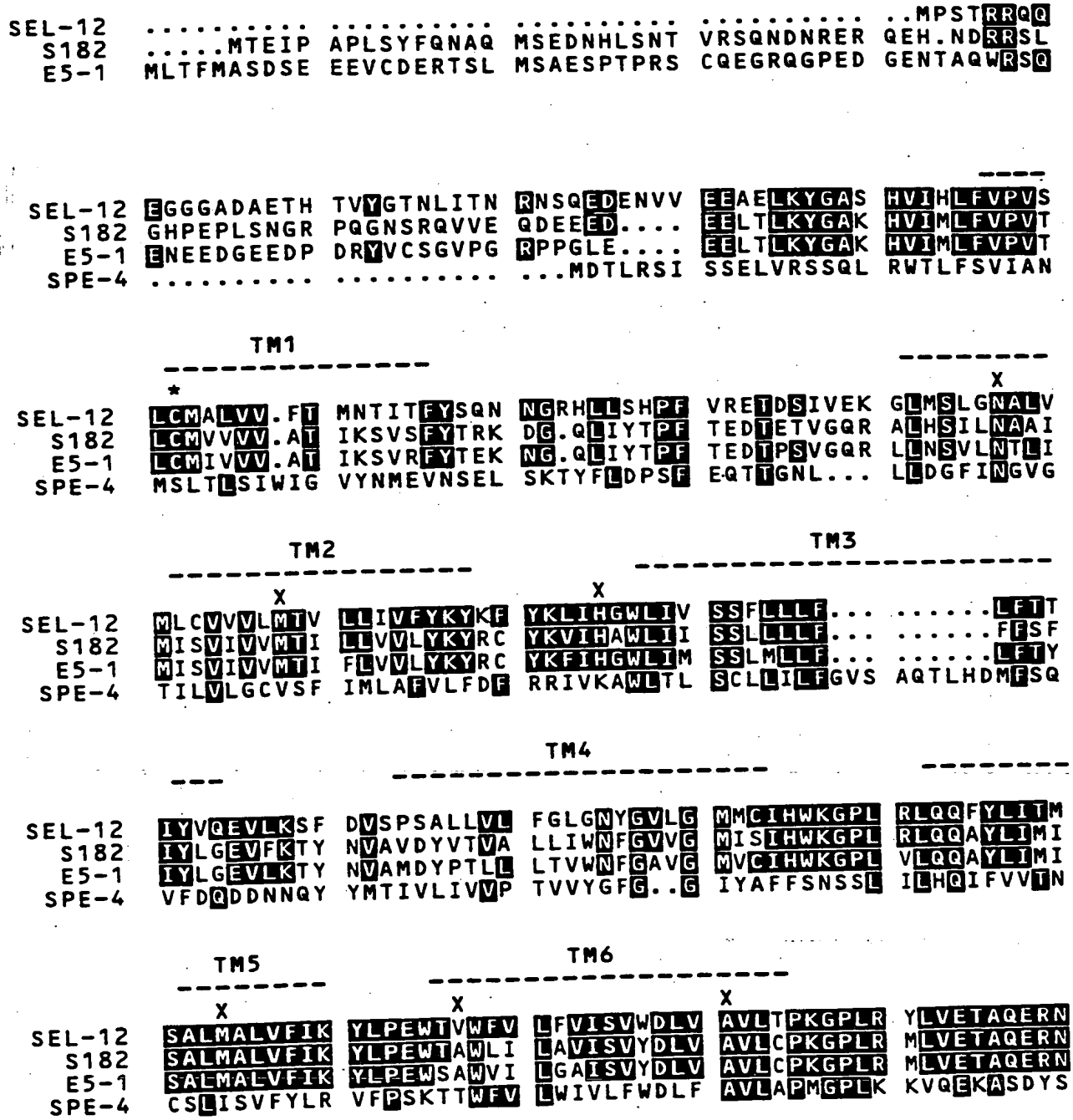
SEL-12 ....MPSTRRQQ
S182 .....MTEIP APLSYFQNAQ MSEDNHLSNT VRSQNDNRER QEH.NDRRSL
E5-1 MLTFMASDSE EEVCDERTSL MSAESPTPRS CQEGRQGPED GENTAQWRSQ
SEL-12 EGGGADAETH TVYGTNLITN RNSQEDENVV EEAELKYGAS HVIHLFVPVS
S182 GHPEPLSNGR PQGNSRQVVE QDEEED.... EELTLKYGAK HVIMLFVPVT
E5-1 ENEEDGEEDP DRYVCSGVPG RPPGLE.... EELTLKYGAK HVIMLFVPVT
SPE-4 ....MDTLRSI SSELVRSSQL RWTLFSVIAN
TM1
X
SEL-12 LCMALVV.FT MNTITFYSQN NGRHLLSHPF VRETDSIVEK GLMSLGNALV
S182 LCMVVVV.AT IKSVSFYTRK DG.QLIYTPF TEDTETVGQR ALHSILNAAI
E5-1 LCMIVVV.AT IKSVRFYTEK NG.QLIYTPF TEDTPSVGQR LLNSVLNTLI
SPE-4 MSLTLSIWIG VYNMEVNSEL SKTYFLDPSF EQTTGNL... LLDGFINGVG
TM2
TM3
X
X
SEL-12 MLCVVVLMTV LLIVFYKYKF YKLIHGWLIV SSFLLLF... ......LFTT
S182 MISVIVVMTI LLVVLYKYRC YKVIHAWLII SSLLLLF... ......FFSF
E5-1 MISVIVVMTI FLVVLYKYRC YKFIHGWLIM SSLMLLF... ......LFTY
SPE-4 TILVLGCVSF IMLAFVLFDF RRIVKAWLTL SCLLILFGVS AQTLHDMFSQ
TM4
SEL-12 IYVQEVLKSF DVSPSALLVL FGLGNYGVLG MMCIHWKGPL RLQQFYLITM
S182 IYLGEVFKTY NVAVDYVTVA LLIWNFGVVG MISIHWKGPL RLQQAYLIMI
E5-1 IYLGEVLKTY NVAMDYPTLL LTVWNFGAVG MVCIHWKGPL VLQQAYLIMI
SPE-4 VFDQDDNNQY YMTIVLIVVP TVVYGFG..G IYAFFSNSSL ILHQIFVVTN
TM5
TM6
X
X
X
SEL-12 SALMALVFIK YLPEWTVWFV LFVISVWDLV AVLTPKGPLR YLVETAQERN
S182 SALMALVFIK YLPEWTAWLI LAVISVYDLV AVLCPKGPLR MLVETAQERN
E5-1 SALMALVFIK YLPEWSAWVI LGAISVYDLV AVLCPKGPLR MLVETAQERN
SPE-4 CSLISVFYLR VFPSKTTWFV LWIVLFWDLF AVLAPMGPLK KVQEKASDYS

FIGURE 2B

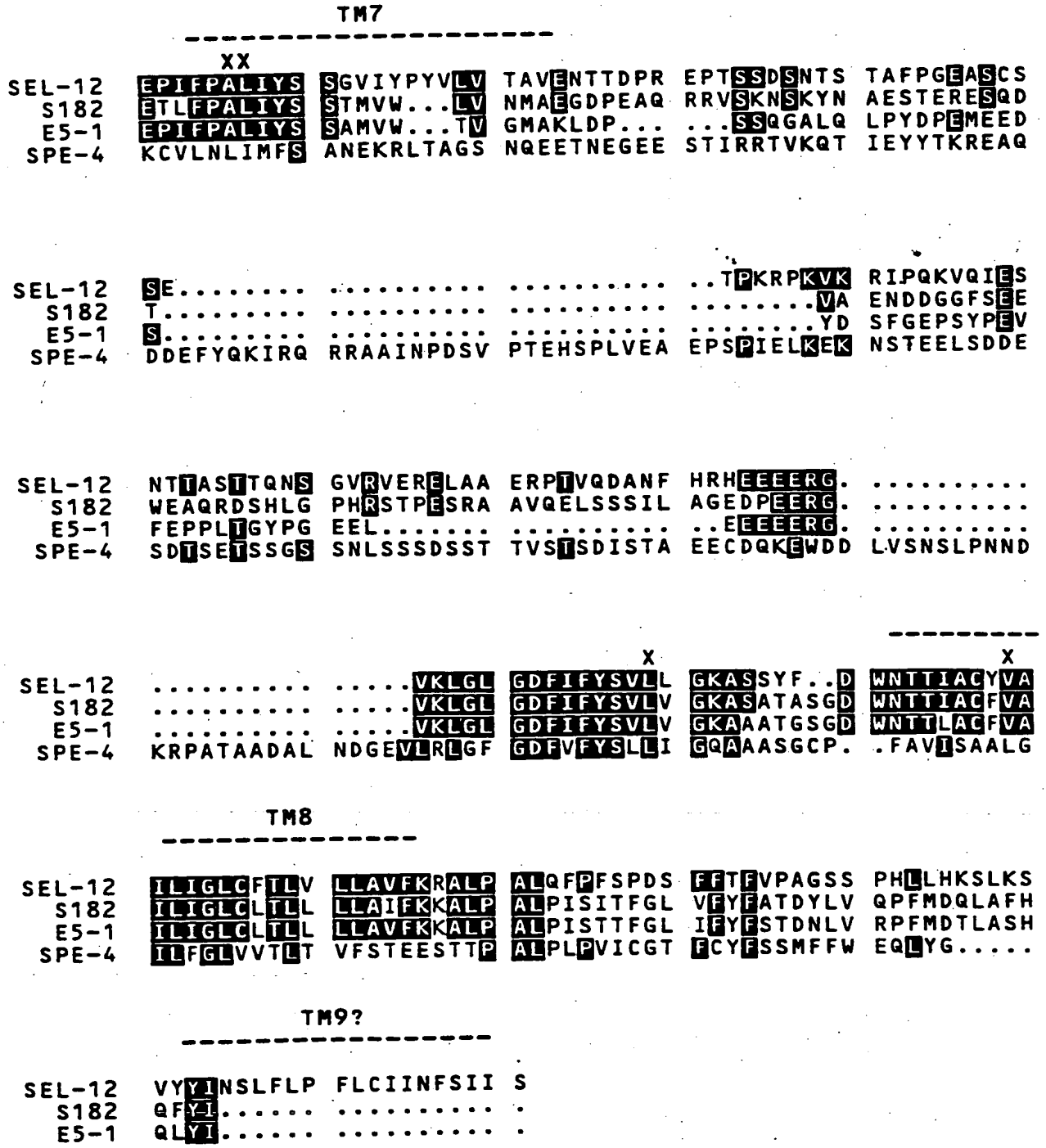
TM7
XX
SEL-12 EPIFPALIYS SGVIYPYVLV TAVENTTDPR EPTSSDSNTS TAFPGEASCS
S182 ETLFPALIYS STMVW...LV NMAEGDPEAQ RRVSKNSKYN AESTERESQD
E5-1 EPIFPALIYS SAMVW...TV GMAKLDP... ...SSQGALQ LPYDPEMEED
SPE-4 KCVLNLIMFS ANEKRLTAGS NQEETNEGEE STIRRTVKQT IEYYTKREAQ
SEL-12 SE..... ....TPKRPKVK RIPQKVQIES
S182 T..... ....VA ENDDGGFSEE
E5-1 S..... ....YD SFGEPSYPEV
SPE-4 DDEFYQKIRQ RRAAINPDSV PTEHSPLVEA EPSPIELKEK NSTEELSDDE
SEL-12 NTTASTTQNS GVRVERELAA ERPTVQDANF HRHEEEERG. .....
S182 WEAQRDSHLG PHRSTPESRA AVQELSSSIL AGEDPEERG. .....
E5-1 FEPPLTGYPG EEL..... ....EEEERG. .....
SPE-4 SDTSETSSGS SNLSSSDSST TVSTSDISTA EECDQKEWDD LVSNSLPNND
X X
SEL-12 ....VKLGL GDFIFYSVLL GKASSYF..D WNTTIACYVA
S182 ....VKLGL GDFIFYSVLV GKASATASGD WNTTIACFVA
E5-1 ....VKLGL GDFIFYSVLV GKAAATGSGD WNTTLACFVA
SPE-4 KRPATAADAL NDGEVLRLGF GDFVFYSLLI GQAAASGCP. .FAVISAALG
TM8
SEL-12 ILIGLCFTLV LLAVFKRALP ALQFPFSPDS FFTFVPAGSS PHLLHKSLKS
S182 ILIGLCLTLL LLAIFKKALP ALPISITFGL VFYFATDYLV QPFMDQLAFH
E5-1 ILIGLCLTLL LLAVFKKALP ALPISTTFGL IFYFSTDNLV RPFMDTLASH
SPE-4 ILFGLVVTLT VFSTEESTTP ALPLPVICGT FCYFSSMFFW EQLYG.....
TM9?
SEL-12 VYYINSLFLP FLCIINFSII S
S182 QFYI..... ..... .
E5-1 QLYI..... ..... .

FIGURE 3A

FIGURE 3B

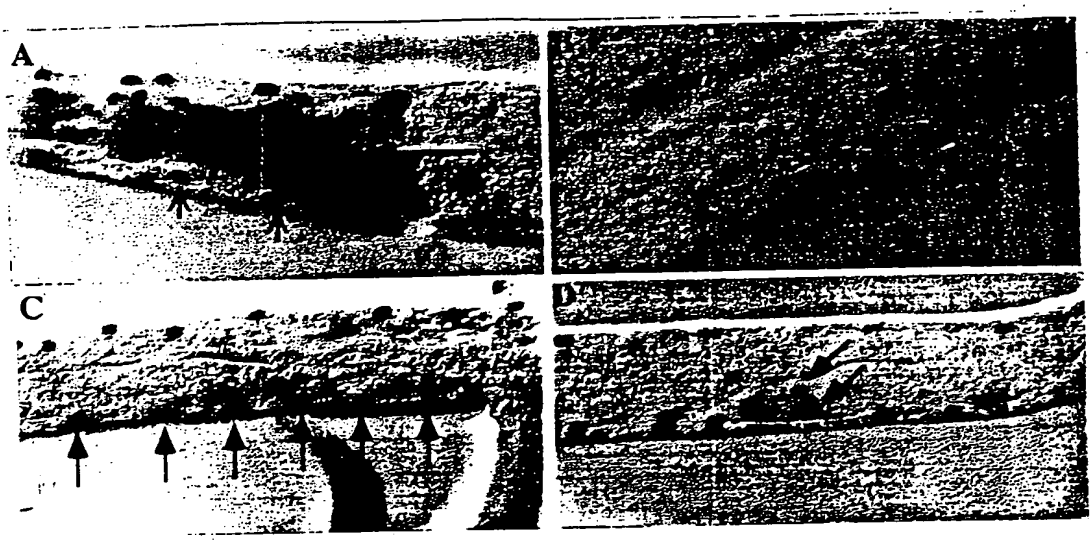

FIGURE 3C

FIGURE 3D

TENT COOPERATION TREATY

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From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION OF PRIORITY DOCUMENTS

(PCT Administrative Instructions, Section 411)

T :

WHITE, John, P.
Cooper & Dunham LLP
1185 Avenue of the Americas
New York, NY 10036
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year) 18 November 1996 (18.11.96)	
Applicant's or agent's file reference 48231-A-PCT	**IMPORTANT NOTIFICATION**
International application No. PCT/US96/15727	**International filing date** (day/month/year) 27 September 1996 (27.09.96)
Priority date (day/month/year) 27 September 1995 (27.09.95)	
Applicant THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK et al	

The applicant is hereby notified of the date of receipt by the International Bureau of the priority document(s) relating to the following application(s):

Priority application No:	Priority date:	Priority country:	Date of receipt of priority document:
60/004,387	27 Sep 1995 (27.09.95)	US	15 Nov 1996 (15.11.96)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

A. Karkachi

Telephone No.: (41-22) 730.91.11

001298554

PATENT COOPERATION TREATY

Columbia

From the INTERNATIONAL SEARCHING AUTHORITY

To: JOHN P. WHITE
COOPER & DUNHAM LLP
1185 AVENUE OF THE AMERICAS
NEW YORK, NY 10036

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

	Date of Mailing *(day/month/year)* 21 JAN 1997
Applicant's or agent's file reference 48231-A-PCT	FOR FURTHER ACTION See paragraphs 1 and 4 below
International application No. PCT/US96/15727	International filing date *(day/month/year)* 27 SEPTEMBER 1996
Applicant THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK	

1. [X] The applicant is hereby notified that the international search report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the international search report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in rules 90 *bis* 1 and 90 *bis* 3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from th priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer CHRISTOPHER S. F. LOW Telephone N . (703) 308-0196

Form PCT/ISA/220 (January 1994)*

(See notes on accompanying sheet)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 48231-A-PCT	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/US96/15727	International filing date *(day/month/year)* 27 SEPTEMBER 1996	(Earliest) Priority Date *(day/month/year)* 27 SEPTEMBER 1995
Applicant THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (See Box I).

2. [X] **Unity of invention is lacking** (See Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] transcribed by this Authority.

4. With regard to the **title**,
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. ____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/15727

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-21, 65

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/15727

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07H 21/04, 21/02; C12N 15/12, 15/70, 1/21
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	LEVITAN et al. Facilitation of lin-12 -mediated signalling by sel-12, a Caenorhabiditis elegans S182 Alzheimer's disease gene. Nature. 28 September 1995, Vol. 377, pages 351-354, see entire document.	1, 2, 4-17, 20, 21, and 65
Y		3, 18, and 19
Y	STRATAGENE CLONING SYSTEMS CATALOG, issued 1993, La Jolla, CA pages 27, 31,2 and 313, see entire document.	18 and 19
Y	SUNDARAM et al. Suppressors of a lin-12 Hypomorph Define Genes That Interact With Both lin-12 and glp-1 in Caenorhabditis elegans. Genetics. November 1993, Vol. 135, pages 765-783, see entire document.	1-21 and 65

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
18 DECEMBER 1996

Date of mailing of the international search report
21 JAN 1997

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
CHRISTOPHER S. F. LOW
Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/15727

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X – Y	Database dbEST, Release 072795, 27 July 1995, National Center for Biothechnology Information, National Library of Medicine, National Institutes of Health, GenBank Accession number H19012, Name H19012, ENTREZ Document Retrieval System, Release 18.0, 15 August 1995, see entire document.	12, 13 ——— 14, 15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/15727

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

536 / 23.5, 24.31, 24.33, 25.1
435 / 320.1, 252.3

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

536 / 23.5, 24.31, 24.33, 25.1
435 / 320.1, 252.3

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

Automated Patent System - USPAT, JPOABS, EPOABS
DIALOG FILES - 5, 11, 73, 76, 144, 155, 156, 185, 434, 440, 444, and 636
Search Terms - sel-12, Caenorhabditis elegans, suppress, enhance, ar131, ar133, and ar171

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To: JOHN P. WHITE
COOPER & DUNHAM LLP
1185 AVENUE OF THE AMERICAS
NEW YORK, NY 10036

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

	Date of Mailing *(day/month/year)* 21 JAN 1997
Applicant's or agent's file reference 48231-A-PCT	FOR FURTHER ACTION See paragraphs 1 and 4 below
International application No. PCT/US96/15727	International filing date *(day/month/year)* 27 SEPTEMBER 1996
Applicant THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK	

1. [X] The applicant is hereby notified that the international search report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the international search report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with th applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in rules 90 *bis* 1 and 90 *bis* 3, respectively, before th completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of th ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer CHRISTOPHER S. F. LOW T lephone No. (703) 308-0196

Form PCT/ISA/220 (January 1994)*

Applicants : Iva Greenwald and E. Jane Hubbard
Serial No. : 08/899,578
Filed : July 24, 1997
Exhibit B

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Articl 18 and Rules 43 and 44)

Applicant's or agent's file reference 48231-A-PCT	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/US96/15727	International filing date *(day/month/year)* 27 SEPTEMBER 1996	(Earliest) Priority Date *(day/month/year)* 27 SEPTEMBER 1995
Applicant THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (See Box I).

2. [X] **Unity of invention is lacking** (See Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] transcribed by this Authority.

4. With regard to the **title**,
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing f this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. _____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes th invention.
   - [X] None of the figures.

INTERNATIONAL SEARCH REPORT

[illegible]ational application N .
PCT/US96/15727

Box I Observations where certain claims were found unsearchable (Continuation f item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-21, 65

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

[redacted]national application No.
PCT/US96/15727

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07H 21/04, 21/02; C12N 15/12, 15/70, 1/21
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	LEVITAN et al. Facilitation of lin-12 -mediated signalling by sel-12, a Caenorhabiditis elegans S182 Alzheimer's disease gene. Nature. 28 September 1995, Vol. 377, pages 351-354, see entire document.	1, 2, 4-17, 20, 21, and 65
Y		3, 18, and 19
Y	STRATAGENE CLONING SYSTEMS CATALOG, issued 1993, La Jolla, CA pages 27, 31,2 and 313, see entire document.	18 and 19
Y	SUNDARAM et al. Suppressors of a lin-12 Hypomorph Define Genes That Interact With Both lin-12 and glp-1 in Caenorhabditis elegans. Genetics. November 1993, Vol. 135, pages 765-783, see entire document.	1-21 and 65

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
18 DECEMBER 1996

Date of mailing of the international search report
21 JAN 1997

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized fficer
CHRISTOPHER S. F. LOW
Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

[redacted]national application No.
PCT/US96/15727

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, f the relevant passages	Relevant to claim No.
X – Y	Database dbEST, Release 072795, 27 July 1995, National Center for Biothechnology Information, National Library of Medicine, National Institutes of Health, GenBank Accession number H19012, Name H19012, ENTREZ Document Retrieval System, Release 18.0, 15 August 1995, see entire document.	12, 13 ——— 14, 15

Form PCT/ISA/210 (continuation of second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/15727

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

536 / 23.5, 24.31, 24.33, 25.1
435 / 320.1, 252.3

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

536 / 23.5, 24.31, 24.33, 25.1
435 / 320.1, 252.3

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

Automated Patent System - USPAT, JPOABS, EPOABS
DIALOG FILES - 5, 11, 73, 76, 144, 155, 156, 185, 434, 440, 444, and 636
Search Terms - sel-12, Caenorhabditis elegans, suppress, enhance, ar131, ar133, and ar171